"Signs, Steven" <sas@lubrizol.com> on 02/13/2001 01:55:09 PM

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Subject: Revision of AMPS(R) Category Test Plan and Robust Summaries

Christie Whitman, Administrator
US Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attention: Chemical Right-to-Know Program, AR-201

Re: Revision of Test Plan and Robust Summaries for the AMPS® Category (CAS # 15214-89-8 and CAS # 5165-97-9) in response to EPA comments

Registration Number:

Dear Administrator Whitman,

The Lubrizol Corporation is pleased to submit the attached revised Test Plan and Robust Summaries for the AMPS® Category of HPV challenge chemicals. These documents were revised in response to EPA comments dated December 27, 2000.

<<AMPS HPV Test Plan.doc>> <<AMPS Category Robust Summary.doc>>

The following revisions were made:

* Addition of robust summaries for transportation/distribution modeling and atmospheric oxidation
* Revision of algal toxicity robust summary
* Correction of Test Plan Table 2 and Table 7 for consistency with hydrolysis robust summary
* Correction of Test Plan for consistency with water solubility robust summary
* Revision of mammalian cell cytogenetics test (1991) robust summary to include mitotic indices
* Revision of mouse micronucleus assay (1996) robust summary to include PCE/NCE ratio results
* Revise fish and invertebrate robust summaries to include temperature and dissolved oxygen content where appropriate

With the submission of these revisions, we anticipate that the AMPS® Category case will be closed.

Please respond with verification that the Agency received this submission.

If there are any questions about this submission, please contact me.

Sincerely,

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 - AMPS HPV Test Plan.doc

 - AMPS Category Robust Summary.doc

AR 201-12958A

High Production Volume (HPV) Challenge Program

Test Plan

For

AMPS@ Category

Prepared by

The Lubrizol Corporation
29400, Lakeland Blvd.
Wickliffe, OH-44092

Date

August 1, 2000

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1 .O INTRODUCTION

On March 2, 1999, the Lubrizol Corporation committed to provide basic toxicity information on chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. The sponsored chemicals addressed in this test plan are:

2-acrylamido-2-methylpropanesulfonic acid (CASRN 152 14-89-8), and

2-acrylamido-2-methylpropanesulfonic acid, sodium salt (CASRN 5 165-97-9).

By participating in this voluntary program, The Lubrizol Corporation agreed to assess the adequacy of existing data; design and submit test plans to fill data gaps; provide test results as they are generated; and prepare summaries of the data characterizing each chemical.

The HPV Challenge Program encourages the development of chemical categories as a mechanism to achieve an efficient completion of the program goals. Based upon the guidance issued by the EPA for the composition of a chemical category, and the inherent structural similarities of the HPV chemicals addressed in this test plan, it was determined that 2-acrylamido-2-methylpropanesulfonic acid and 2-acrylamido-2-methylpropanesulfonic acid, sodium salt are ideal candidates for review under a single chemical category. For the purposes of this test plan and the accompanying robust summaries, the chemical group will be referred to as the AMPS@ category. In support of the AMPS@ category approach, modeling and test data for 2-acrylamido-2-methylpropanesulfonic acid, ammonium salt (CASRN 58374-69-9; a non-HPV chemical) are included in the test plan and robust summaries.

To ease the task of communicating information on the members of the AMPS® category in this test plan, the following abbreviated chemical names will be used:

AMPS® acid (2-acrylamido-2-methylpropanesulfonic acid),

Sodium AMPS@ (2-acrylamido-2-methylpropanesulfonic acid, sodium salt);

Ammonium AMPS@ (2-acrylamido-2-methylpropanesulfonic acid, ammonium salt)

2.0 EVALUATION OF DATA

The EPA guidance on chemical categories states that structural similarities among members of a category create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and human health effects. As will be evident from the information provided below, the data findings for the members of the AMPS@ category fulfill these criteria and support the inclusion of the acid and its neutral salts as a single chemical family. Consequently, it is valid that data from one or more AMPS@ derivatives is used to satisfy a particular environmental, aquatic or health-related endpoint.

The process of evaluating the basic toxicity of the members of the AMPS@ chemical category entailed the following stepwise process: grouping of chemicals into a putative category; gathering relevant data for each member of the category; evaluating the compiled data for adequacy; evaluation of the physico-chemical, environmental, aquatic and health-effects data to confirm the correlation between category members; construction of a matrix of SIDS endpoints for the category members; and identification of data gaps for critical endpoints within the AMPS@ category (Table 7; illustration of the matrix).

2.1 Physical Chemical Description of the AMPS@ Category

A chemical category is defined by EPA for the purposes of the HPV Chemical Challenge Program as a group of chemicals whose physico-chemical and toxicological properties are likely to be similar, or to follow a regular pattern as a result of structural similarity. The members of the AMPS@ category are virtually homologous, characterized by a 2-acrylamido-2-methylpropanesulfonic parent anion, distinct only by the corresponding H^+, Na^+ or NH_4^+ counterion (Figure 1).

AMPS® acid is prepared by reacting acrylonitrile, isobutylene, and oleum in the presence of water. The sodium AMPS@ and ammonium AMPS® salts are subsequently formed by neutralization of AMPS@ acid with sodium hydroxide or ammonium hydroxide, respectively. The AMPS@ monomer (parent structure) is a propanesulfonic acid substituted at the C2 position with a methyl group and an acrylamido moiety. Reactive sites on the molecule include the unsaturated vinyl group and the terminal sulfonic acid.

The molecular weight of AMPS@ acid is 207, whereas the molecular weight of sodium AMPS® and ammonium AMPS@ are 229.2 and 224.2, respectively. The melting point for ammonium AMPS® was estimated using the capillary tube method to be 191°C. The melting point for the AMPS@ acid and sodium AMPS@ was estimated to be 160.78°C and 260.35°C, respectively using the EPIWIN' model (EPA and Syracuse Research Corporation). The AMPS@ monomer decomposes at, or slightly above, it's melting point temperature. As a result, boiling point determinations are not applicable. Vapor pressure estimations were performed using analytical techniques and mathematical modeling. Gravimetric testing methods estimated the vapor pressure of ammonium AMPS® to be 7.4×10^{-9} Pa @ 25°C. Mathematical modeling using the EPIWIN estimation software estimated the vapor pressure of AMPS@ acid and sodium AMPS® to be $6.75 \times 1\ 0^{-9}$ and 1.72×10^{-13} mm Hg at 25°C respectively. Due to the low vapor pressure, the members of this category will not significantly volatilize into the vapor phase. The octanol-water partition coefficient ($\log_{10}P_{ow}$) for ammonium AMPS@ was experimentally determined to be -3.41 @ 22°C. Partition coefficient for the AMPS@ acid and sodium AMPS@ was estimated to be -2.19 and -4.34 respectively, using the EPIWIN estimation program. AMPS® monomers are hygroscopic and hydrophilic. The water solubility of the ammonium AMPS@ acid was experimentally determined to be 76 gm/1 00 gm water whereas the EPIWIN model projected the water solubility of AMPS@

[1] EPIWIN. Estimation program Interface for Windows, Version 3.02. Syracuse Research Corporation, Syracuse, NY, USA

acid and sodium AMPS@ to be is approximately 100 gm/100 gm water and 150 gm/100 gm water, respectively.

2.2 Exposure Information for the AMPS@ Category

AMPS@ acid is manufactured and supplied as a white crystalline solid. The sodium and ammonium salts of AMPS@ monomer are prepared as 50% aqueous solutions. AMPS® monomer is primarily used for the preparation of high molecular weight water-soluble polymers. AMPS@ monomer can be polymerized in solution using conventional vinyl moiety polymerization. AMPS@ is available as a crystalline solid or as an aqueous salt solution. AMPS@ monomer is a highly reactive, hydrophilic sulfonic acid acrylic monomer capable of imparting a number of distinctive high-performance characteristics to a wide variety of ion-containing polymers and reaction products. The earliest patents using AMPS® monomer were filed for acrylic fiber manufacturing. Oil drilling, water treatment and coating/adhesive applications soon followed. Today, there are over 1,000 patents and publications involving manufacture and use of AMPS@ monomer, representing a diversity of applications including cosmetics, medical technolo9gies, electrodeposition/plating, fuel and lubricant additives, flocculants, ion exchange resins, non-woven binders/adsorbents, photographic chemicals, paper, dispersants and textiles. AMPS® monomer can be polymerized in solution using conventional vinyl polymerization techniques. AMPS® monomer is low to hydrolyze in both its monomer and homopolymer configurations. As a result, AMPS® monomer imparts exceptional hydrolytic stability to resulting polymers.

2.3 Environmental Fate Data

Computer modeling techniques were used to evaluate the environmental fate and transport for members of the AMPS@ category. If experimental data was not available, the physical-chemical properties of the AMPS® category members were estimated by using the EPIWIN model. The AMPS@ acid was used as the prototype chemical for estimating the physical-chemical properties of this category. As stated above, the members of this category are characterized by a 2-acrylamido-2-methylpropanesulfonic parent anion, distinct only by the corresponding H^+, Na^+ or NH_4^+ counterion. For environmental fate estimations, the physical chemical characteristics of the acid is an appropriate read-across for the sodium and ammonium AMPS@ salts included in this category. The physical-chemical properties (experimental and modeled data) of AMPS@ acid are provided in Table 1A.

2.3.1 Fugacity Modeling

Fugacity is a thermodynamic term used to describe the behavior of a chemical in the environment. Fugacity-based multimedia modeling compares the relative distribution of chemicals between environmental compartments (i.e., air, soil, water, suspended sediment, sediment and biota). A widely used model for this approach is the Equilibrium Criterion Model[2] (EQC). There are 3 levels of the EQC model and EPA has recommended its use in the document titled, *"Determining the Adequacy of Existing*

[2] Mackay, et al. Evaluating the environmental fate of a variety of types of chemicals using the EQC Model. Environ. Toxicol. Chem. 15: 1627-1637

Data". In this document, EPA states that it accepts Level 1 fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes basic physical-chemical properties including molecular weight, vapor pressure, octanol-water partition coefficient and water solubility to calculate percent distribution within a standardized regional environment. The EQC Level II model also calculates the rates of transport and degradation within the environmental compartment. Application of the Level II model requires data on the rates of biodegradation, hydrolysis, photolysis and oxidation. EQC Level III evaluates the effects of discharge rates to air, water and soil and inter-media transport rates.

The EQC Level I model was used to estimate the relative distribution of AMPS@ category chemicals among environmental compartments. The AMPS@ acid was used as the representative chemical in the level 1 model. Results of the EQC Level 1 modeling shows that the AMPS® category members would partition almost exclusively into the water phase (Table 1B). Based on the high water solubility coupled with low soil adsorption and volatility, it is expected that chemicals in this group will partition preferentially into the water phase.

2.3.2 Hydrolysis

Hydrolysis is a chemical transformation process in which an organic molecule reacts with water, forming a new carbon-oxygen bond and cleaving a carbon-X bond in the original molecule, where X is the leaving group. Hydrolytic reactions depend on the susceptibility of the chemical to attack by a nucleophile such as a water molecule or hydroxide ion. Molecules that are susceptible to hydrolysis are those in which the electron distribution gives some charge separation, facilitating nucleophilic attack. The lack of a suitable leaving group renders compounds resistant to hydrolysis. Potentially hydrolyzable groups include esters, carbamates, epoxides, halomethanes and selected alkyl halides.

The potential for the AMPS@ Category chemicals to undergo hydrolysis could not be evaluated using the EPIWIN model due to lack of hydrolyzable functional groups. However, the AMPS@ monomer contains an amide functional group that could potentially hydrolyze into a carboxylic acid and an amine. In general, amides are much less hydrolytically reactive than esters and hydrolysis half-lives can range from hundreds to thousands of years in the aquatic environment. Shelf-live determinations indicated that aqueous solutions of sodium AMPS® at pH 9 resist hydrolysis for months under normal storage. All three members of the AMPS@ category are structurally similar and can therefore be expected to exhibit similar hydrolysis characteristics. The hydrolytic stability likely results from the gem-dimethyl substitution adjacent to the amide group. Aqueous solutions of 2-acrylamido-2-methylpropanesulfonic anion will eventually hydrolyze to acrylic acid and dimethyltaurine. Based on the information available, the members of this category will not undergo significant hydrolysis and no additional testing is required.

2.3.3 Biodegradation

Biodegradation refers to transformation of chemicals by microorganisms where parts of the chemical are incorporated into cellular material or used as an energy source for the organisms, and the remainder is converted to simple inorganic molecules. The complete biodegradation of a substance to CO_2, H_2O and simple organic and inorganic end products is called mineralization. Biodegradability tests vary from those that only measure primary degradation (i.e., any biologically induced structural change in the parent compound) or ultimate degradation (i.e., complete conversion to inorganic compounds such as CO_2, H_2O; or methane if anaerobic microorganisms are involved). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals by infrared absorbance, or by a chemical-specific method. Ultimate degradation (also called mineralization) is determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on the elemental analysis.

Biodegradation can be measured using the OECD or U.S. EPA test guidelines. The biodegradation results for the compounds in this category are summarized in Table 2. A biodegradation test was conducted on AMPS® acid and sodium AMPS@ using the semi-continuous activated sludge (SCAS) method (40CFR 795.3340). In the 44-day test, the biodegradation rate of both test materials was <10% based on dissolved organic carbon measurements. Based on the test results, both these compounds exhibited a very slow rate of biodegradability and are not readily biodegradable.

A Modified Sturm Test (OECD 301B) was conducted on ammonium AMPS@. In 28 days, 3.3% of the test material was converted to CO_2. Consequently, it was also assessed as exhibiting a very slow rate of biodegradability. Based on available data, all members of the category can be characterized as having a very slow rate of biodegradability and no additional tests are required.

2.3.4 Photodegradation

Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation. Environmental photo-reactions take place in the presence of sunlight only above 295nm in the near ultraviolet (UV) extending into the infrared region (750 nm) of the electromagnetic spectrum. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. The presence of O_3 in the troposphere leads to the formation of OH radicals through the photolysis of O_3 at wavelengths ~ 290 to 350 mn. The OH radical is the key reactive species in the troposphere, reacting with all organic compounds apart from chlorofluorocarbons (CFCs) and other halogenated compounds which do not contain a hydrogen atom.

The tendency of the AMPS@ acid to photodegrade was evaluated by using the modeling program EPIWIN which includes calculation of atmospheric oxidation potential (AOP). This computer simulation of photo-oxidation is recommended in the Agency's recently released structure activity review (SAR) guidance for HPV chemicals. This program

calculates chemical half-life based on an overall OH reaction rate constant, a 12-hour day, and a given OH concentration. The model results are provided below:

Overall OH Rate Constant = 16.3284 x 10^{-12} cm^3/molecule-sec
Half life = 0.655 days (12-hr day; 1.5 x 10^6 OH/cm^3)

All three members of the AMPS@ category are structurally similar and can therefore be expected to exhibit similar photodegradation characteristics. Based on the high water solubility and estimated low vapor pressure for members of this category, atmospheric oxidation is not likely to be a significant degradation pathway.

2.4 ECOTOXICOLOGY DATA

The purpose of the acute toxicity tests is to evaluate effects that occur rapidly as a result of short-term exposure to a chemical. Generally, acute effects are relatively severe, the most common one measured in aquatic organisms is lethality. A chemical is considered acutely toxic if by its direct action it causes mortality in 50% or more of the exposed population in a relatively short period of time, such as 96 hours to 14 days. Factors that may directly influence the toxicity of a chemical include solubility, vapor pressure, pH and lipophilicity. The chemicals in the AMPS® category are soluble in water, however, their low lipophilicity (as indicated by the low octanol water partition coefficient values) precludes significant bioconcentration in aquatic organisms. Available toxicity data summarized in Table 3 indicates that members of this category are not highly toxic to aquatic species.

2.4.1 Acute Fish Toxicity

Acute fish toxicity tests were conducted for both the AMPS@ acid and sodium AMPS@ in accordance with EPA-660/3-75-009 over a 96-hour exposure period. The median lethal concentration (LC50) was estimated to be 170 mg/L with the AMPS® acid and >1,000 with sodium AMPS@. Sublethal effects at 600 and 1 ,000-mg/L test solution was observed with the AMPS@ acid whereas no effects were seen with sodium AMPS@.

An LC50 value of 1,400 mg/L was derived in a 96-hour fish toxicity test conducted with ammonium AMPS@ in accordance with OECD 203. The test results suggest that the AMPS@ acid is more toxic to fish than the corresponding salt. The acute fish toxicity data available for the AMPS@ category chemicals is adequate and no further testing is required.

2.4.2 Acute Invertebrate Toxicity

Acute invertebrate toxicity tests were conducted for both the AMPS® acid and sodium AMPS@ in accordance with EPA-660/3-75-009 over a 48-hour exposure period. The median effects concentration (EC50) was estimated to be 340 mg/L with the AMPS@ acid and > 1,000 with sodium AMPS@.

An EC50 value of 1,200 mg/L was derived in a 48-hour invertebrate toxicity test conducted with ammonium AMPS@ in accordance with OECD 202. The test results suggest that the AMPS@ acid is more toxic to the test organism than the corresponding

salt. The acute invertebrate toxicity data available for the AMPS@ category chemicals is adequate and no further testing is required.

2.4.3 Algae Acute Toxicity

Acute algal toxicity test was conducted with ammonium AMPS@ in accordance with OECD 201 over a 96-hour exposure period. The median effects concentration (EC50) was estimated to be >2,000 mg/L, the highest test concentration. Algal toxicity data is not available for the AMPS@ acid or sodium AMPS@. Based on the structural similarity of the members of this category and relatively low toxicity, the data for ammonium AMPS® will be bridged to AMPS@ acid and sodium AMPS®.

2.4.4 Summary of the Acute Aquatic Effects of Members of the AMPS@ Category

Results of the acute toxicity tests show that the members of the AMPS@ category are not significantly toxic to aquatic species. The LC50 and EC50 values in the fish and invertebrate toxicity tests respectively, were higher than 100 mg/L with all three members of this category. The AMPS@ acid was slightly more toxic to the test organisms in both fish and invertebrate tests, compared to the sodium and ammonium AMPS®. In tests conducted with algae, the EC50 value for ammonium AMPS@ was greater than 2,000 mg/L. Based on available data, it is apparent that the 2-acrylamido-2-methylpropanesulfonic parent anion present in all 3 members of this category, is not significantly toxic to aquatic organisms. The algae data available for ammonium AMPS® will be bridged to other members of this group and no additional testing is required.

2.5 HUMAN HEALTH TOXICOLOGICAL DATA

2.5.1 Acute Health Effects of the AMPS@ Category

Acute health effects test results are summarized in Table 4.

2.5.1 .1 Acute Oral Toxicity

Acute oral toxicity studies in rats are available for all three members of the AMPS@ category. The acute oral toxicity studies presented were performed in accordance with OECD guidelines (OECD 401). The acute oral LD50 for AMPS@ acid was 1830 mg/kg. As is reported in the robust summary for AMPS® acid, animal deaths were reported only at doses greater than 2000 mg/kg (range of doses 500-8000 mg/kg). Principle clinical findings following high oral doses of this strong acid were consistent with oral administration of a severe gastrointestinal irritant. In contrast, acute oral LD50s for the neutral salts of AMPS@ monomer (i.e., sodium AMPS@ and ammonium AMPS@) were greater than 5000 mg/kg. No unscheduled deaths were recorded after the administration of the AMPS@ salts and no remarkable clinical observations were noted in the treated animals. The results of all three studies were deemed reliable without restriction according to the Klimisch criteria.

2.5.1.2 Acute Dermal Toxicity

The acute dermal toxicity studies were performed in accordance with OECD guidelines (OECD 402). These studies were performed in rabbits using ammonium AMPS@. The acute dermal LD50 was determined to be >2000 mg/kg. No unscheduled deaths were recorded in the study and no remarkable clinical observations or gross pathological findings suggestive of adverse systemic effects were noted. No preferential dermal toxicity was demonstrated. The results of this study were deemed reliable without restriction according to the Klimisch criteria.

2.5.1.3 Summary of the Acute Toxicological Effects of the AMPS@ Category

Results of the acute toxicity studies indicate that the parent 2-acrylamido-2-methylpropanesulfonic anion does not exhibit direct systemic toxicity via the oral or dermal routes of administration. This is evidenced by the high acute oral LD50 seen with experiments using the neutral sodium and ammonium salts of AMPS@ monomer, and the high LD50 observed following prolonged dermal application of ammonium AMPS®. The lower LD50 and adverse clinical findings associated with the oral administration of AMPS® acid are attributed to its strongly acidic properties resulting in severe local gastrointestinal reactions and the resulting secondary adverse physiological responses, Therefore, these acute toxicity results support the direct relationship between the physico-chemical properties and the acute toxicological findings for the members of the AMPS@ category. Since all members of the group likely have an equally low level of toxicity under acute conditions, no additional testing is required.

2.5.2 Genetic Toxicology of the AMPS@ Category

Genetic toxicity test results are summarized in Table 5.

2.5.2.1 Bacterial Gene Mutation Assay

In vitro reverse mutation assays using AMPS@ acid were performed in six different strains of bacteria in accordance with international guidelines (combined OECD 47 1 and 472). Mutagenicity was studied with metabolic activation and in non-activated conditions. The conclusion of two separate investigations was that AMPS® acid was non-mutagenic under the conditions of the assay. The results of this study were deemed reliable without restriction according to the Klimisch criteria.

2.5.2.2 Mammalian Gene Mutation Assay

In vitro assays were performed in Chinese Hamster Ovary (CHO) cells to study the ability of AMPS@ acid to induce mutations at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus. The methods used were consistent with OECD guideline 476. The test material was tested with, and without, metabolic activation. In two separate studies, no significant increase in the frequency of mutagenic events was detected in cells treated with AMPS@ acid. The conclusion of these studies was that AMPS@ acid was not mutagenic in mammalian systems. The results of one study was deemed reliable without restriction (Klimisch criteria) whereas the results of the other study were reliable with restrictions due to the lack of a metabolic activation test as recommended in OECD guidelines.

2.5.2.3 *In vitro* Chromosomal Aberration Assay

An in *vitro* assay was performed in Chinese hamster ovary (CHO) cells to study the ability of AMPS@ acid to induce chromosomal aberrations. The method used was consistent with OECD guideline 473. The test material was tested with, and without, metabolic activation. The test material did not induce chromosomal aberrations in the non-activated assay, however, AMPS® acid exposed to hepatic microsomal activation produced non-dose-dependent increases in the frequency of chromosomal aberrations. The conclusion of this study was that metabolic activation of AMPS@ acid may result in clastogenic activity. However, the absence of a dose-response effect, absence of a time-response effect, lack of reproducibility between experiments, coupled with the simultaneous occurrence of excessive cytotoxicity with the observed clastogenic events confounds the interpretation of the positive findings. The results of these studies were deemed reliable without restriction according to the Klimisch criteria. To further elucidate this potential, AMPS@ acid was tested for the potential to produce chromosomal aberrations in an *in vivo* assay system.

2.5.2.4 *In* vivo Chromosomal Aberration Assay

In vivo assays were performed in two distinct assays in two species of mammals to study the ability of AMPS® monomer to induce chromosomal aberrations. A mouse micronucleus assay was performed according to OECD guideline 474. The clastogenic effect of intraperitoneal injection of AMPS@ acid in male and female mice at doses ranging from 175-1750 mg/kg was measured at 24, 48 and 72 hours. These treatments did not produce statistically significant or dose-dependent increases in the frequency of chromosomal aberrations. In a further study, ammonium AMPS@ was tested in a rat bone marrow cytogenetics test (OECD guideline 475). At doses ranging from 150-l 500 mg/kg, oral administration of ammonium AMPS@ to male and female rats did not produce statistically significant or dose-dependent increases in the frequency of chromosomal aberrations. The combined results of these tests indicate that AMPS@ monomer is not clastogenic under *in vivo* assay conditions. The results of these studies were deemed reliable without restriction according to the Klimisch criteria.

2.5.2.5 Summary of the Genetic Toxicolonv of the AMPS@ Category

Results of the genetic toxicity testing on AMPS@ acid clearly show that the parent 2-acrylamido-2-methylpropanesulfonic anion is not a mutagen. Repeated negative findings in bacterial both and mammalian *in vitro* assay systems support this conclusion. The results of the single *in vitro* chromosomal aberration are inconclusive. Specifically, the clastogenic effects observed with the metabolic activation of AMPS@ acid were not replicable and did not follow dose-responsive or time-responsive patterns. Furthermore, the clastogenic events were associated with excessive cytotoxic damage, and therefore may not represent direct genotoxic activity. Consequently, *in vivo* tests in rats and mice were performed to more reliably describe the cytogenetic toxicity of AMPS@. These rodent assays clearly demonstrate the absence of clastogenic potential resulting from the *in* vivo administration of AMPS@ acid or ammonium AMPS® in mammalian systems. In conclusion, 2-acrylamido-2-methylpropanesulfonic anion is not considered to be a clastogen. Since AMPS@ acid, sodium AMPS® and ammonium AMPS® are simple salts of the same parental anion, the lack of mutagenicity or *in vivo* clastogenic activity can be

extrapolated to the entire AMPS@ category. Moreover, these combinations of genetic toxicity results further support the direct relationship between the physico-chemical properties and the toxicological findings for the entire membership of the AMPS@ category. Since all members of the group likely have an equally low index of genetic toxicity, no additional testing is required.

2.5.3 Repeated Dose Health Effects of the AMPS@ Category

Repeated dose health effect test results are summarized in Table 6.

2.5.3.1 Subchronic Oral Toxicitv

A 2%day repeated dose oral toxicity test in rats was performed on ammonium AMPS@. This study was completed in accordance with OECD guideline 407. Five groups of male and female rats received doses of ammonium AMPS® ranging from 0 (water vehicle control) to 1000 mg/kg/day for 28 consecutive days, followed by a 14-day treatment-free recovery period. The were no deaths in the study, and the most remarkable clinical sign was gastrointestinal unrest manifested by lethargy, emaciation, diarrhea and reduced food consumption in a single male at the highest dose. Otherwise, there were no treatment-related untoward effects on clinical observations, body weight, food consumption, serum chemistry values, hematology values, gross pathological observations or histopathological findings. As a result, the laboratory study director assigned the no-observed-effect-level (NOEL) at 1000 mg/kg/day. The result of this study was deemed reliable without restriction according to the Klimisch criteria. The data in this study are reflective of a low subchronic toxicity index for ammonium AMPS@. In light of the strong physico-chemical and toxicological similarity between the members of the AMPS® category, this low index of subchronic toxicity can be applied to the category as a whole.

2.5.3.2 Reproductive/Developmental Toxicitv

An assessment of the reproductive and developmental toxicity of ammonium AMPS@ was performed using the method prescribed in OECD guideline 42 1. In this reproductive/developmental screen, male and female rats were dosed for two weeks prior to mating and, in the case of the females, through gestation, parturition and lactation day 4. Oral administration of the test material at doses of 100, 500 and 1000 mg/kg/day had no effect on FO survival, growth, mating behavior, copulation, fertility, precoital intervals, gestation lengths, corpora lutea counts, implantation counts, mean live litter size, pre- or post-implantation loss, gross necropsy findings or organ weights (testes and epididymides). Histopathological examination of the testes, ovaries and epididymides from control and high-dose rats did not reveal any test material-related microscopic changes. No test material-related effects were observed in the F1 offspring with respect to survival, clinical observations, body weights or gross necropsy findings. In addition, there were no indications of test material-related developmental effects in the F1 pups at any dosage level tested. Based on the results of this study, the laboratory study director assigned both the reproductive NOEL and the developmental NOEL at 1000 mg/kg/day. The results of this study were deemed reliable without restriction according to the Klimisch criteria. This data indicated that ammonium AMPS@ has a low index of reproductive or developmental toxicity. Based on the physico-chemical and toxicological

similarity of the members of the AMPS@ category, this low index of reproductive/ developmental toxicity can be applied to all members of the AMPS® category.

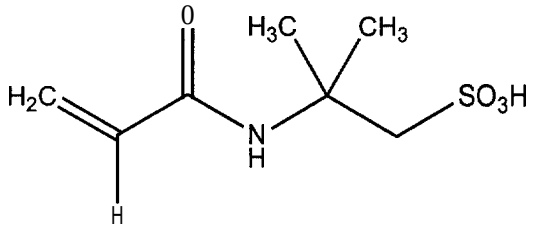
253.3 Summary of the Repeated Dose Toxicity data for the AMPS@ Category

Results of the oral repeated dose toxicity testing on ammonium AMPS® clearly show that the parent 2-acrylamido-2-methylpropanesulfonic anion is not a cumulative or a reproductive/developmental toxicant. Repeated negative findings at high doses in both a subchronic repeated dose study and a reproductive/ developmental screening assay support this conclusion. Therefore, these repeated dose toxicity results support the direct relationship between the physico-chemical properties and the acute toxicological findings for the members of the AMPS® category. Since all members of the group likely have equally low index of subchronic or reproductive/developmental toxicity under repeated dose conditions, no additional testing of AMPS@ acid or sodium AMPS@ is required.

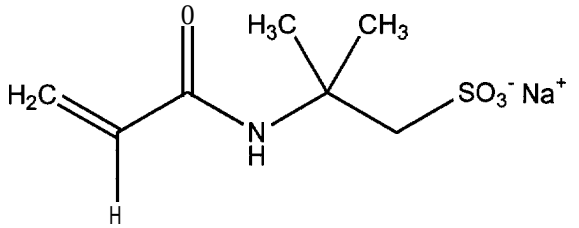
3.0 CONCLUSIONS

Physical-chemical, environmental fate, ecotoxicology and health-effects toxicity data (experimental or computer modeled) for the members of the AMPS® category has been evaluated for adequacy. The available data is of high quality and projects a profile of low overall toxicity. The consistency of the data between AMPS® acid, sodium AMPS@ and ammonium AMPS® for these endpoints supports the AMPS® category approach, As a result, the use of conservative read-across within the category for existing data gaps will preclude any further testing.

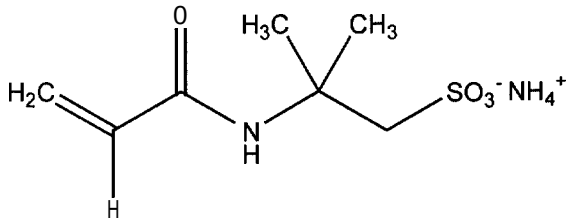
Figure I- Chemical Structures of the AMPS® Category



2-acrylamido-2-methylpropanesulfonic acid
CASRN 15214-89-8
AMPS@ acid



2-acrylamido-2-methylpropanesulfonic acid, sodium salt
CASRN 5 165-97-g
Sodium AMPS@



2-acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN 58374-69-9
Ammonium AMPS®

Table IA: Physical Properties and Environmental Fate Data for AMPS@ acid

CAS #	Molecular Weight	Log K_{ow}	Water Solubility (mg/L)	Vapor Pressure (mm Hg)	Log K_{oc}	Log BCF	Melting Point (°C)	Atmospheric Oxidation	
								OK Rate Constant (cm^3/molec-sec)	Half-life (hrs)
15214-89-8	207.25	-2.19	10^6	6.75E-09	1.0	0.5	160.78	16.3E-12	7.8

Table 1B: Predicted Environmental Distribution of AMPS® acid

CAS#	Air (%)	Water (%)	Soil (%)	Sediment (%)	Suspended Sediment (%)	Biota (%)	Fugacity (Pa)
15214-89-8	3.7E-09	100	5.7E-04	1.27E-05	3.97E-07	5.48E-03	4.5E-16

Table 2 - Evaluation of Environmental Fate Data for the AMPS@ Category

CHEMICAL	BIODEGRADABILITY		HYDROLYSIS		PHOTODEGRADATION	
	AVAILABLE DATA	PROPOSED TESTING	AVAILABLE DATA	PROPOSED TESTING	AVAILABLE DATA	PROPOSED TESTING
AMPS@ acid (CAS #15214-89-8)	Adequate data (<10% biodegraded after 44 days)	No testing needed- Adequate data	No data available	No testing needed- Bridging	EPI WIN Model Estimation (Half life = 0.655 days [12-hr day; 1.5 x 10^6 OH/cm^3])	No testing needed - Adequate data
Sodium AMPS@ (CAS #5 165-97-9)	Adequate data (<10% biodegraded after 44 days)	No testing needed- Adequate data	Yes (Resistant to hydrolysis)	No testing needed- Adequate data	No data available	No testing needed- Bridging
Ammonium AMPS@ (CAS #58374-69-9)	Adequate data (<3.3% biodegraded after 28 days)	No testing needed- Adequate data	No data available	No testing needed- Bridging	No data available	No testing needed- Bridging

Table 3 – Evaluation of Aquatic Toxicity Data for the AMPS@ Category

CHEMICAL (CAS #)	ACUTE TOXICITY TO FISH[1]			ACUTE TOXICITY TO INVERTEBRATES[2]			ACUTE TOXICITY TO ALGAE[3]		
	AVAILABLE DATA		PROPOSED TESTING	AVAILABLE DATA		PROPOSED TESTING	AVAILABLE DATA		PROPOSED TESTING
	96-hour LC_{50} (mg/L)	96-hour NOEC (mg/L)		48-hour EC_{50} (mg/L)	48-hour NOEC (mg/L)		96-hour EC_{50} (mg/L)	96-hour NOEC (mg/L)	
AMPS® acid (CAS #15214-89-8)	170	130 (LC0)	No testing needed – Adequate data	340	78 (LC0)	No testing needed – Adequate data	No data available	No data available	No testing needed - Bridging
Sodium AMPS@ (CAS #5165-97-9)	>1,000	1,000 (LC0)	No testing needed – Adequate data	>1,000	1,000 (LC0)	No testing needed Adequate data	No data available	No data available	No testing needed Bridging
Ammonium AMPS@ (CAS #58374-69-9)	1,400	640 (LCO)	No testing needed Adequate data	1,200	640 (LCO)	No testing needed – Adequate data	>2,000	2,000 (LC0)	No testing needed – Adequate data

'Species is either Fathead minnow or Bluegill.
'Species is *Daphnia magna* unless otherwise noted.
[3]Species is freshwater algae *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum)* unless otherwise noted.
[4]LC0 indicates concentration where no mortality or effects were seen.

Table 4 – Evaluation of Human Health Acute Toxicity Data for the AMPS@ Category

	ACUTE ORAL TOXICITY		ACUTE DERMAL TOXICITY	
CHEMICAL (CAS #)	AVAILABLE DATA	PROPOSED TESTING	AVAILABLE DATA	PROPOSED TESTING
AMPS® acid (CAS #15214-89-8)	LD_{50} = 1830 mg/kg	No testing needed – Adequate data	No data available	No testing needed – Bridging
Sodium AMPS® (CAS #5165-97-9)	LD_{50} > 16,000 mg/kg	No testing needed – Adequate data	No data Available	No testing needed – Bridging
Ammonium AMPS® (CAS #58374-69-9)	LD_{50} > 5000 mg/kg	No testing needed – Adequate data	LD_{50} > 2000 mg/kg	No testing needed – Adequate data

Table 5 – Evaluation of Human Health Genetic Toxicity Data for the AMPS® Category

	Bacterial Mutagenicity		Mammalian Mutagenicity		*In vitro* chromosomal aberration		*In vivo* chromosomal aberration	
CHEMICAL (CAS #)	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**	**AVAILABLE DATA**	**PROPOSED TESTING**
AMPS® acid (CAS #15214-89-8)	Non-mutagenic	No testing needed – Adequate data	Non-mutagenic	No testing needed – Adequate data	Suspicion of clastogenicity	No testing needed – Adequate data	Non-clastogenic	No testing needed – Adequate data
Sodium AMPS® (CAS #5165-97-9)	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging
Ammonium AMPS® (CAS #58374-69-9)	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging	No data available	No testing needed – Bridging

Table 6– Evaluation of Human Health Repeated Dose Toxicity Data for the AMPS@ Category

	28-DAY REPEATED DOSE TOXICITY		REPRODUCTIVE/DEVELOPMENTAL TOXICITY	
CHEMICAL (CAS #)	AVAILABLE DATA	PROPOSED TESTING	AVAILABLE DATA	PROPOSED TESTING
AMPS® acid (CAS #15214-89-8)	No data Available	No testing needed – Bridging	No data Available	No testing needed – Bridging
Sodium AMPS® (CAS #5165-97-9)	No data Available	No testing needed – Bridging	No data Available	No testing needed – Bridging
Ammonium AMPS@ (CAS #58374-69-9)	NOEL = 1000 mg/kg/day	No testing needed – Adequate data	F0 NOEL = 1000 mg/kg/day F1 NOEL = 1000 mg/kg/day	No testing needed – Adequate data

Table 7 – Test Plan for AMPS@ Category

	AMPS@ acid	Sodium AMPS@	Ammonium AMPS®
Physico-Chemical			
Melting Point	**X**[m]	**X**[m]	X
Boiling Point	NA	NA	NA
Vapor Pressure	**X**[m]	**X**[m]	X
Partition Coefficient	**X**[m]	**X**[m]	X
Water Solubilitv	**X**[m]	**X**[m]	x
Environmental Fate			
Photodegradation	**X**[m]	x	x
Hydrolysis	x	x	**X**
Fugacity	**X**[m]	x	x
Biodegradation	X	X	X
Ecological Toxicity			
Acute Fish	X	X	X
Acute Al&e	x	x	**X**
Acute Daphnia	**X**	**X**	**X**
Mammalian Toxicity			
Acute Health (oral &/or dermal)	X	**X**	**X**
Bacterial Mutagenicity	X	x	x
Mammalian Mutagenicity	**X**	x	x
In vitro Cytogenetics	**X**	x	x
In vivo Cytogenetics	X	x	**X**
Repeated Dose Toxicity	x	x	**X**
Repro/Developmental Toxicity	x	x	**X**

Where:

X – Actual test data
x – Read-across from other members of the AMPS® category
X[m] – Modeling data
NA – Endpoint not applicable for chemical

PHYSICO-CHEMICAL PROPERTY – MELTING POINT	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Remarks	This substance is referred to as Ammonium AMPS@ in the test plan for the AMPS@ category
Method	
Method/Guideline followed	Testing was conducted as specified in Commission Directive 92/69/EEC
Test Type	Melting Point
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Melting point deter&nation was carried out using the Capillary Method/Melting Temperature Devices with Liquid Bath, Method A 1 of Commission Directive 92/69/EEC.
Results	The test material was determined to melt at 191°C with decomposition above 228°C.
Data Quality	Reliable without restrictions – Klimisch Code 1
References	OS 114452: Determination of General Physico-Chemical Properties, SafePharm Laboratories LTD., 12/7/95.
Other:	

PHYSICO-CHEMICAL PROPERTY - OCTANOL/WATER PARTITION COEFFICIENT	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS#	*58374-69-9*
Remarks	This substance is referred to as Ammonium AMPS® in the test. plan for the AMPS@ category
Method	
Method/Guideline followed	Testing was conducted according to method A8 specified in Commission Directive 92/69/EEC
Test Type	Partition coefficient
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Octanol/water partition coefficient was determined by measuring the amount of test material (5 mg/L aqueous solution) that distributed into n-octanol (water-saturated) after 5 min of flask shaking at 22°C. Test material distributed into the n-octanol phase was measured using high performance liquid chromatography and UV detection.
Results	The octanol/water partition coefficient of the test material was determined to be 3.87 x 10^{-4} at 22°C. $Log_{10}P_{ow}$ = -3.41
Data Quality	Reliable without restrictions - Klimisch Code 1
References	OS 114452: Determination of General Physico-Chemical Properties, SafePharm Laboratories LTD., 12/7/95.
Other:	

PHYSICO-CHEMICAL PROPERTY – SOLUBILITY	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Remarks	This substance is referred to as Ammonium AMPS® in the test plan for the AMPS@ category
Method	
Method/Guideline followed	Testing was conducted according to method A6 specified in Commission Directive 92/69/EEC
Test Type	Water solubility
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks field for test conditions	Water solubility was determined by measuring the amount of solid test article that distributed into distilled water after 17 hours of flask shaking at 20°C. The solubilized test material was measured using high performance liquid chromatography and UV detection.
Results	The water solubility of the test material was determined to be 761 g/l at 20°C.
Data Quality	Reliable without restrictions – Klimisch Code 1
References	OS 114452: Determination of General Physico-Chemical Properties, SafePharm Laboratories LTD., 12/7/95.
Other:	

PHYSICO-CHEMICAL PROPERTY – PHOTODEGRADATION	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	
Method/Guideline followed	EPIWIN (The Atmospheric Oxidation Potential (AOPWIN) module was used)
Test Type	Atmospheric Oxidation
GLP (Y/N)	N
Year (Study Performed)	2000
Remarks field for test conditions	EPIWIN. Estimation program Interface for Windows, Version 3.02. Syracuse Research Corporation, Syracuse, NY, USA
Results	The overall OH Rate Constant = 16.3284 x 10^{-12} cm-'/molecule-sec. Half life = 0.655 days (12-hr day; 1.5 x 10^{6} OH/cm^{3}).
Data Quality	Reliable without restrictions – Klimisch Code 1
References	Unpublished confidential business information
Other:	*Copyright 2000 The Lubrizol Corporation. AMPS® is a registered trademark of The Lubrizol Corporation. The information contained in this robust summary may not be published, broadcast, rewritten or otherwise distributed without the prior written authority of The Lubrizol Corporation.*

PHYSICO-CHEMICAL PROPERTY – HYDROLYSIS	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	
Method/Guideline followed	Potentiometric titration
Test Type	Hydrolysis
GLP (Y/N)	N
Year (Study Performed)	1978
Remarks field for test conditions	The hydrolysis of AMPS monomer sodium salt at pH=12 creates sodium acrylate and ß, ß-dimethyltaurine. The hydrolysis was done at 50°C, with 631 ppm 4-methoxyphenol and at 80°C with 506 ppm of 4-methoxyphenol. The hydrolysis was followed by potentiometric titration with perchloric acid in glacial acetic acid. Sodium acrylate, a strong base, and ß, ß-dimethyltaurine, a weak base were measured.
Results	At 50°C, there was only about 6% hydrolysis in 7 days. At 80°C, the half-life of the hydrolysis was about 5 days.
Data Quality	Reliable with restrictions – Klimisch Code 1
References	Unpublished confidential business information
Other:	

PHYSICO-CHEMICAL PROPERTY – HYDROLYSIS	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5 165-97-9
Remarks	This substance is referred to as Sodium AMPS® in the'test plan for the AMPS@ category
Method	
Method/Guideline followed	Potentiometric titration
Test Type	Hydrolysis
GLP (Y/N)	N
Year (Study Performed)	1978
Remarks field for test conditions	AMPS monomer is a strong acid. The hydrolysis of AMPS monomer at pH=1 creates two weak acids, acrylic acid and ß, ß-dimethyltaurine. The hydrolysis was done at 50°C, with 63 1 ppm of 4-methoxyphenol to inhibit polymerization, and at 80°C with 506 ppm of 4-methoxyphenol. The hydrolysis was followed by the potentiometric titration of the solution with tetrabutylammonium hydroxide in t-butyl alcohol. Endpoints for both the strong acid, AMPS, and for the weak acids, acrylic acid and ß, ß-dimethyltaurine were observed. The degree of hydrolysis was calculated from the ratio of strong acid to weak acid.
Results	At 50°C, hydrolysis was negligible. At 80°C, the half-life of the hydrolysis was about 7 days.
Data Quality	Reliable with restrictions – Klimisch Code 1
References	Unpublished confidential business information
Other:	

PHYSICO-CHEMICAL PROPERTY – FUGACITY	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	
Method/Guideline followed	EQC (Equilibrium Criterion Model, Level 1)
Test Type	Fugacity
GLP (Y/N)	N
Year (Study Performed)	2000
Remarks field for test conditions	Mackay, et al. Evaluating the environmental fate of a variety of types of chemicals using the EQC Model. Environ. Toxicol. Chem. 15: 1627- 1637
Results	The EQC level 1 model predicted that the AMPS@ acid is going to partition exclusively (100%) into the aqueous phase.
Data Quality	Reliable without restrictions – Klimisch Code 1
References	Unpublished confidential business information
Other:	

PHYSICO-CHEMICAL PROPERTY – VAPOR PRESSURE	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	5 8374-69-9
Remarks	This substance is referred to as Ammonium AMPS@ in the test plan for the AMPS@ category
Method	
Method/Guideline followed	Testing was conducted according to method A4 specified in Commission Directive 92/69/EEC
Test Type	Vapor pressure
GLP (Y/N)	Y
Year (Study Performed)	1995
Remarks "field for test conditions	Vapor pressure' was determined using a vapor pressure microbalance with measurements being made at several temperatures. Linear regression analysis was used to calculate the vapor pressure.
Results	The vapor pressure of the test material was determined to be 7.4 x 10^{-9} Pa at 25°C
Data Quality	Reliable without restrictions – Klimisch Code 1
References	OS 114452: Determination of Vapor Pressure, SafePharm Laboratories LTD., 10/16/95.
Other:	

AQUATIC TOXICITY – FISH	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS@ in the test plan for the AMPS@ category
Method	
Method/Guideline followed	"Methods for acute toxicitytests with fish, macroinvertebrates, and amphibians", EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain/Supplier	Bluegill (*Lepomis macrochirus*)
Analytical Monitoring	None
Exposure Period (unit)	96 hours
Statistical Methods	$LC50$ values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	Test Organisms: Source – In house laboratory supply (Lot # 83A13); Age- Juvenile; Length – 3.8 cm; Wet weight – 0.66 g; Loading rate – n/a; Pretreatment – none, fish were acclimated to the test conditions for 14 days prior to start of test Test System: The static acute test was conducted using nominal test concentrations of 130, 220, 360, 600 and 1000 mg/L. A stock solution (150 mg/ml) of the test substance was prepared and separately dispersed in each test vessel to yield the definitive test concentrations. The test was conducted glass exposure vessels that contained 15 L of test solution. 10 fish were used for each test concentration (no replicates were used). The test solution was not aerated and maintained on a photoperiod of 16 hours light and 8 hours darkness. The fish were not fed during the test Dilution Water: Source – soft water reconstituted from deionized water; Hardness Water adjusted to a hardness of 44 mg/L as CaC03; Alkalinity – 32 mg/L; Analysis – n/a; Water chemistry in test: DO (mg 02/L) – 5.5 to 9.2; pH 7.5; specific conductance – 140 µmhos/cm Test Temperature (°C) – 22 ± 1 Test Levels: Control, 130, 220, 360, 600 and 1000 mg/L
Results	*96-hour LC50 = >1000 mg/L based on nominal test concentrations*
Remarks	Measured concentration: n/a

	Unit: mg/L 96-hour LCO = 1000 mg/L Statistical results: 96-hour LC50 = >1000 mg/L. LCO (0 – 96 hours) = 1000 mg/L Other: • Effect concentrations based on nominal loading rates • No mortality or sublethal effects were seen at the highest test concentration of 1000 mg/L.
Conclusions	96-hour LC50 = >1000 mg/L. 96-hour LCO = 1000 mg/L
Data Quality	*Reliable with restrictions- Klimisch Code 2*
References	*Acute Toxicity of OS # 489331" to Bluegill (Lepomis macrochirus). Bionomics study # -043-0983-H77- IOO; Bionomics report # BW-83-l I-1491. EG&G Bionomics, November, 1983*
Other:	

AQUATIC TOXICITY – INVERTEBRATE	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5 165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS® in the test plan for the AMPS@ category
Method	
Method/Guideline followed	"Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study P & f o r m e d)	1983
Species/Strain	***Cladoceran***, Daphnia magna
Analytical Monitoring	N/a
Exposure Period (unit)	48 hours
Statistical methods	EC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: A stock solution (10 mg/ml) of the test substance was prepared in distilled water. An appropriate amount of the stock solution was then added to the appropriate amount of dilution water to yield 1000 ml. Each test solution was divided into 3 beakers to provide replicates containing 200 ml each. The test solution was not aerated during the exposure period. Daphnids were not fed during the test and maintained on a 16-hour light and 8-hour dark photoperiod. Dilution water: Well water was fortified according to the test protocol and filtered through a carbon filter and an Amberlite ZAD-7 resin column to remove any potential organic contaminants. The dilution water had a hardness of 160 - 180 mg/L as CaC03. Analysis – n/a; Water chemistry in test: DO- 7.8 – 9.3 mg/L; pH – 8.0 – 8.3; specific conductance – 400 - 600 µmhos/cm. Test Temperature ("C): 20 ± 1 Element: Immobilization/mortality Test Levels: Test concentrations included control, 130,220, 360, 600and 1000 mg/L. Fifteen daphnia were used per test group (5 per replicate).
Results	48-h EC_{50} = >1000 mg/L. 48-h EC_0 = 1000 mg/L
Remarks	Measured concentration: n/a Unit: mg/L

	Other: • Effect concentrations based on nominal loading rates. • No mortality or sublethal effects were seen at the highest test concentration of 1000 mg/L
Conclusions	48-h EC_{50} = >1000 mg/L. 48-h EC_0 = 1000 mg/L
Data Quality	*Reliable with restrictions- Klimisch Code 2*
References	*Acute Toxicity of OS # 48933F* to *Daphnia magna. Bionomics study # - 043-0983-H77-1 IO; Bionomics report # B W-83-l I-14901. EG&G Bionomics, November, 1983*
Other:	

ENVIRONMENTAL FATE – BIODEGRADATION	1
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CAS #	5165-97-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS@ in the test plan for the AMPS@ category
Method	
Method/Guideline followed	The Evaluation of the biodegradation of test materials using the semi-continuous activated sludge method. (40 CFR 795.3340)
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)'	1986
Contact time (units)	44 days
Inoculum	Secondary activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	Inoculum: Four liters of secondary activated sludge were collected from a domestic wastewater treatment plant. Raw sewage collected from the primary settling tank was used as the influent during acclimation and test periods. Concentration of test chemical: From day 1 to 8, 20-mg carbon/L influent was introduced into the test unit. This was reduced to IO-mg carbon/L from day 8. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium Test Setup: Aeration units consisted of glass vessels with cone shaped lower ends. The units contain 150 ml of mixed liquor when filled. A drain hole is located at the 50 ml level to facilitate sampling and removal of effluent. The acclimation process included aeration for 23 hours, settling of sludge, removing 100 ml of supernatant and resuming aeration to 500 mg/minute. The units were fed raw sewage daily till a clear supernatant liquor was obtained. At the end of the acclimation period, sludge from all units was pooled and mixed. Adequate amount was added to each unit to achieve a suspended solids concentration of 1500 mg/L for each unit. One-mg carbon/100 ml influent was added to the appropriate test units. Blank units did not receive any test material. The study was extended to 44 days to determine if acclimation and biodegradation will increase in this time frame. Sampling frequency: Samples were collected for DOC analysis on all workdays. Controls: Yes; blank unit was included.

	Analytical method: DOC analysis was conducted after sparging the samples with nitrogen prior to analysis to remove soluble carbon dioxide. Method of calculating measured concentrations: N/A Other:
Results Degradation % after time Kinetic (for sample, positive and negative controls) Breakdown Products (Y/N) If yes describe breakdown products Remarks	<10% after 44 days. n/a
Conclusions	The test substance showed a low biodegradation rate (<10%) in *28* days.
Data Quality	*Reliable with restrictions- Klimisch Code 2*
References	*Evaluation of the biodegradation of OS 69793F using the modified SCAS methodfor Lubrizol Corporation. Ref: 86-0843-11; Hill Top Research Inc., Nov 12, 1986.*
Other:	

AQUATIC TOXICITY – FISH	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS	# 58374-69-g
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS@ in the test plan for the AMPS@ category
Method	
Method/Guideline followed	OECD 203 (1992)
Test Type	Static renewal (test media was renewed every 24 hours)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain/Supplier	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring	The concentration and stability of the test material in the test solutions were verified by HPLC analysis at 0, 24 and 96 hours. Analysis of the test solutions at 0, 24 and 96 hours showed the measured test concentrations to be near nominal and so the results are based on nominal test concentrations.
Exposure Period (unit)	96 hours
Statistical Methods	LC50 values and associated confidence limits were calculated by the moving average method of Thompson (1947). Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. BACT Reviews, 11, pages 115-145.
Remarks field for test conditions	Test Organisms: Source – Neil Hardy Aquatica, Carshalton, Surrey, UK; Age- Juvenile; Length – 3.8 cm; Wet weight – 0.54 g; Loading rate – 0.27 g/L; Pretreatment – none, fish were acclimated to the test conditions for 12 days prior to start of test Test System: The static acute test was conducted using nominal test concentrations of 200, 360, 640, 1120 and 2,000 mg/L. The test substance was separately dispersed in 20 litres of dechlorinated tap water to yeild the definitive test concentrations. The test was conducted glass exposure vessels that contained 20 L of test solution. 10 fish were used for each test concentration (no replicates were used). The test vessels were convered to reduce evaporation and maintained on a photoperiod of 16 hours light and 8 hours darkness. The test vessels were aerated via narrow bore glass tubes. Test media were renewed daily. The fish were not fed during the test Dilution Water: Source – Dechlorinated tap water; Hardness – Water adjusted to a hardness of 100 mg/L as CaC03; Analysis – Water was free of measurable quantities of pesticides; Water chemistry in test: DO (mg 02/L) – 8.2 to 8.5; pH – 7.4 to 7.7 Test Temperature (°C) – 21 .O. Test Levels: Control, 200, 360, 640, 1120 and 2,000 mg/L
Results	*96-hour LC50 = 1400 mg/L based on nominal test concentrations*
Remarks	Measured concentration: Analysis of the test solutions at 0, 24 and 96

	hours showed the measured test concentrations to be near nominal as shown below: O-hour (% of nominal) 24-hour (% of nominal) 96-hour (% of nominal) Control <LOQ <LOQ <LOQ 200 mg/L 100 99 103 360 mg/L 102 105 101 640 mg/L 101 99 102 1120 mg/L 101 100 102 2000 mg/L 103 102 Where LOQ = Limit of quantitation Unit: mg/L 96-hour LCO = 640 mg/L Statistical results: 72 and 96-hour LC50 = 1400 mg/L. 95% CL (1300-1600) mg/L. Other: • Effect concentrations based on nominal loading rates • No insoluble material was noted • Sublethal effects including loss of equilibrium was seen at 96 hours in 1120 mg/L test concentration and after 3 hours in the 2000 mg/L test concentration.
Conclusions	72 and 96-hour LC50 = 1400 mg/L. 96-hour LCO = 640 mg/L
Data Quality	*Reliable without restrictions- Klimisch Code I*
References	*OS 114454: Acute toxicity to Fathead minnow (pimehales promelas). SPL Project Number: 525/049. SafePharm Laboratories Limited. May 2, I996*
Other:	

AQUATIC TOXICITY – INVERTEBRATE	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS@ in the test plan for the AMPS' category
Method	
Method/Guideline followed	OECD #202 (1984)
Test Type	Static acute toxicity test
GLP (Y/N)'	Y
Year (Study Performed)	1996
Species/Strain	***Cladoceran,*** Dapbnia magna
Analytical Monitoring	The concentration and stability of the test material in the test solutions were verified by HPLC analysis at 0 and 48 hours. Analysis of the test solutions at 0 and 48 hours showed the measured test concentrations to be near nominal and so the results are based on nominal test concentrations.
Exposure Period (unit)	48 hours
Statistical methods	EC50 values and associated confidence limits were calculated by the moving average method of Thompson (1947). Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. BACT Reviews, 11, pages 115-145.
Remarks field for test conditions	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: The test material was prepared by direct dispersion in reconstituted water to give a 4000 mg/L stock solution from which dilutions were made in reconstituted water to prepare the test series. A 250-mL glass beaker that contained 200 mL of test solution was used per treatment. The test solution was not renewed during the exposure period. The test vessels were loosely covered to reduce entry of dust, etc. Daphnids were not fed during the test. Dilution water: The dilution water had a hardness of 270 mg/L as CaC03. Water chemistry in test: DO (mg 02/L) – 7.9 to 8.4; pH – 7.3 to 7.6. Test Temperature (ºC) – 2 1 .0. Element: Immobilization/mortality Test Levels: Test concentrations included control, 36, 64, 112, 200, 360, 640, 1120, 2000 and 3600 mg/L. Ten daphnia were used per test group and each test included replicate vessels.
Results	48-h EC_{50} = 1200 mg/L. 48-h EC_0 = 640 mg/.
'Remarks	Measured concentration: Analysis of the test solutions at 0 and 48 hours showed the measured test concentrations to be near nominal as shown below:

	0-hour (% of nominal) / 24-hour (% of nominal) Control: <LOQ / <LOQ 36 mg/L: 93 / 95 112 mg/L: 95 / 96 360 mg/L: 96 / 100 1120 mg/L: 97 / 98 3600 mg/L: 98 / 99 Where LOQ = Limit of quantitation Unit: mg/L Other: • Effect concentrations based on nominal loading rates. • Control response was satisfactory (100% survival and no sublethal effects).
Conclusions	48-h EC_{50} = 1200 mg/L. 48-h EC,, = 640 mg.
Data Quality	*Reliable without restrictions- Klimisch Code I*
References	***OS 114454: Acute toxicity to Daphnia magna. SPL Project Number: 525/047. SafePharm Laboratories Limited. April 30, 1996***
Other:	

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name CAS #	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt 58374-69-9
Purity: Remarks	50% aqueous solution This substance is referred to as ammonium AMPS® in the test plan for the AMPS@ category
Method	
Method/Guideline followed	OECD 301B
'Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1996
Contact time (units)	2 8 days.
Inoculum	Return activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	Inoculum: The sludge was aerated and stirred for 2 hrs in a flask, homogenized in a Waring blender at low speed for 2 minutes and let stand for 1 hour. The supernatant from the homogenized activated sludge was used as inoculum. The bacterial count in the supernatant was estimated using the Easicult dip-slide. The microbial level in the test mixture was 10,000 cells/mL Concentration of test chemical: A known concentration of test substance was added to the mineral medium, giving at least 30 mg ThOD per L medium. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium Test Setup: Seven flasks were used for the test substance, reference and controls. Each test vessel was connected to a series of 3 absorption bottles, each containing 100 mg of 0.0125 M (0.025N) barium hydroxide solution. The test was started by bubbling CO2-free air through the suspensions at a rate of 50 to 100 ml/min. The test vessels were covered with aluminum foil during the test period to prevent algal growth and photodegradation of test materials. Sampling frequency: During the first 10 days, the analysis of CO2 was made every 2-3 days and then approximately every 5" day until the 28th day. CO2 evolution from the test suspensions and inoculum blanks was followed in parallel. Controls: Yes; blank and positive controls (sodium benzoate), abiotic and toxicity checks were included. Sodium benzoate was used as the positive control Analytical method: For measurement of evolved C02, the barium hydroxide absorber closest to the test vessel was disconnected and titrated with 0.1M HCl using phenolphthalein as the indicator.

	Method of calculating measured concentrations: N/A Other:
Results	
Degradation % after time Kinetic (for sample, positive and negative controls)	3.3% after 28 days. Reference (sodium benzoate) – > 60% degradation in 4 days achieving a total of 93% in 28 days Abiotic degradation – 4.99% in 28 days, indicating insignificant physiochemical degradation of the test substance Toxicity check – Combined degradation of 48% for the reference and test substances, indicating the test substance was not toxic to the microbial populations in the inoculum.
Breakdown Products (Y/N) If yes describe breakdown products R e m a r k s	N
Conclusions	The test substance showed a low biodegradation rate (3.3%) in 28 days. The reference substance, sodium benzoate, reached a level of 93% in the same test period.
Data Quality	*Reliable without restrictions- Klimisch Code I*
References	*OS 114454: Biodegradability study of test substance OS 114454 using the OECD CO2 evolution (Modified Sturm) test. Document NO. 65-95-0199-BC-001. Ricerca Inc. June 18, 1996.*
Other:	

AQUATIC TOXICITY – ALGAE	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CAS #	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS@ in the test plan for the AMPS' category
Method	
Method/Guideline followed	OECD 20 1 (1984)
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	'1996
Species/Strain	***Freshwater alga& Selenastrum capricornutum***
Element basis (# of cells/ml)	10,000 cells/mL
Exposure period/duration	'96 hours
Analytical monitoring	'The concentration and stability of the test material in the test solutions were verified by HPLC analysis at 0 and 96 hours. Analysis of the test solutions at 0 and 96 hours showed the measured test concentrations to be near nominal and so the results are based on nominal test concentrations.
Statistical methods	Statistical analysis of the area under the growth curve data was carried out for the control and 2000 mg/L test groups using Students t-test.
Remarks field for test conditions	Test Species: Cells taken from a log-growth phase in-house culture of *Selenastrum capricornutum* that was originally obtained from the Culture Centre for Algae and Protozoa (CCAP), Institute of Freshwater Ecology, Ferry House, Ambleside, Cambia. Test System: A limit test was conducted at a test concentration of 2000 mg/L. The test material was prepared by a direct dispersion in culture medium and volume adjusted to give a 4000 mg/L stock solution. An aliquot of the stock solution was mixed with 500 ml of algal suspension to give the test concentration of 2000 mg/L. Test Conditions: Six 250-ml conical flasks containing 100-mL of solution were prepared for the treatment group and 3 flasks were used for the control. At study initiation, the culture contained nominal cell density of -10,000 cells/ml. The flasks were covered with aluminum foil and incubated at 24 + 1°C, and constantly shaken at 100 rpm for 96 hours. Samples were taken at 0, 24, 48, 72 and 96 hours and the absorbance measured at 665 nm. Daily cell counts were made concurrently with a hemocytometer to confirm that the absorbance values were sufficiently well correlated with cell density values. Light: Cool-white fluorescent lights provided a light intensity of ~ 7000 lux under continuous illumination. Test temperature(°C) – 24 ± 1

Shaker bed speed (rpm): 100

Dilution Water: Sterile enriched alga growth media adjusted to pH 8.0. pH ranged from 8.0 – 8.1 at O-hour and 10.0 – 10.2 after 96 hours.

Test Levels: Control (3 replicates) and 2000 mg/L (6 replicates)

Results

Nominal concentrations: 72-h and 96-h EL_{50}s = >2000 mg/L for both biomass and specific growth

Remarks

The growth was monitored using absorbance values as they correlated well with cell density. The mean cell density and absorbance values in the control and absorbance values in the test concentration are shown below:

	0 hour	24 hour	48 hour	72 hour	96 hour
Control (cells/ml)	1.23×10^4	1.08 x 10'	1.81×10^5	3.33×10^5	1.51×10^6
Control Absorbance	0.022	0.100	0.220	0.529	1.074
Test Conc. (2000 mg/L)	0.022	0.101	0.232	0.523	1.112

Measured concentration: Analysis of the test solutions at 0 and 96 hours showed the measured test concentrations to be near nominal as shown below:

	O-hour (% of nominal)	96-hour (% of nominal)
Control	<LOQ	<LOQ
Treatment (R1 – R3)	96	100
Treatment (R4 – R6)	97	102

Where LOQ = Limit of quantitation

Unit: mg/L

There were statistically no significant differences ($P \geq 0.05$), between the control and 2000 mg/L test groups and therefore the no observed effect concentration (NOEC) is given as ≥ 2000 mg/L.

Other:

- Effect concentrations based on nominal loading rates
- No insoluble material was noted

Control response was satisfactory.

Conclusions

No effects to algae growth or biomass was seen at the test concentration at 2000 mg/L.

Data Quality

Reliable without restrictions- Klimisch Code I

References

OS 114454: Algal Inhibition Test. SPL Project Number: 525/046. SafePharm Laboratories Limited. May 1, 1996

Other:

AQUATIC TOXICITY – FISH	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS@ category
Method	
Method/Guideline followed	"Methods for acute toxicity tests with fish, macro&vertebrates, and amphibians", EPA-66013-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed)	1983
Species/Strain/Supplier	Bluegill *(Lepomis macrochirus)*
Analytical Monitoring	None
Exposure Period (unit)	96 hours
Statistical Methods	LC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	Test Organisms:. Source In house laboratory supply (Lot # 83A13); Age- Juvenile; Length – 3.8 cm; Wet weight – 0.66 g; Loading rate – n/a; Pretreatment – none, fish were acclimated to the test conditions for 14 days prior to start of test Test System: The static acute test was conducted using nominal test concentrations of 130,220, 360, 600 and 1000 mg/L. A stock solution (150 mg/ml) of the test substance was prepared and separately dispersed in each test vessel to yield the definitive test concentrations. The test was conducted glass exposure vessels that contained 15 L of test solution. 10 fish were used for each test concentration (no replicates were used). The test solution was not aerated and maintained on a photoperiod of 16 hours light and 8 hours darkness. The fish were not fed during the test Dilution Water: Source – soft water reconstituted from deionized water; Hardness – Water adjusted to a hardness of 44 mg/L as CaC03; Alkalinity – 32 mg/L; Analysis – n/a; Water chemistry in test: DO (mg 02/L) 5.5 to 9.2; pH 7.5; specific conductance – 140 µmhos/cm Test Temperature (°C) – 22 ± 1 Test Levels: Control, 130, 220, 360, 600 and 1000 mg/L
Results	*96-hour LC50 = 170 mg/L based on nominal test concentrations*
Remarks	Measured concentration: n/a Unit: mg/L

	96-hour LCO = 130 mg/L Statistical results: 72 and 96-hour LC50 = 170 mg/L. 95% CL (130 - 220) mg/L. LCO (0 - 96 hours) = 130 mg/L Other: • Effect concentrations based on nominal loading rates • Sublethal effects including loss of equilibrium and rapid respiration was seen at 0 hours in the 600 and 1000 mg/L test concentration.
Conclusions	72 and 96-hour LC50 = 170 mg/L. 96-hour LCO = 130 mg/L
Data Quality	*Reliable with restrictions- Klimisch Code 2*
References	*Acute toxicity of OS # 26935F to Bluegill (Lepomis macrochirus). Toxicity test report submitted to Lubrizol Corporation. Bionomics Study # 043-0983-H76- IOO; Bionomics Report # B W-83-l I - 1492. EG&G Bionomics, November, 1883.*
Other:	

AQUATIC TOXICITY – INVERTEBRATE	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	
Method/Guideline followed	"Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians", EPA-660/3-75-009
Test Type	Static
GLP (Y/N)	Y
Year (Study Performed).	1983
Species/Strain	***Cladoceran***, Daphnia magna
Analytical Monitoring	n/a
Exposure Period (unit)	48 hours
Statistical methods	EC50 values and associated confidence limits were calculated by using the computer program of Stephan et al. 1978. The statistical methods used included one of the following: moving average, probit analysis and binomial probability.
Remarks field for test conditions	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: A stock solution (10 mg/ml) of the test substance was prepared in distilled water. An appropriate amount of the stock solution was then added to the appropriate amount of dilution water to yield 1000 ml. Each test solution was divided into 3 beakers to provide replicates containing 200 ml each. The test solution was not aerated during the exposure period. Daphnids were not fed during the test and maintained on a 16-hour light and 8-hour dark photoperiod. Dilution water: Well water was fortified according to the test protocol and filtered through a carbon filter and an Amberlite ZAD-7 resin column to remove any potential organic contaminants. The dilution water had a hardness of 160 – 180 mg/L as CaC03. Alkalinity: 110 – 130 mg/L; Analysis -n/a; Water chemistry in test: DO- 7.9 – 9.5 mg/L; pH 7.9 8.3; specific conductance 400 - 600 µmhos/cm. Test Temperature (°C): 20 ± 1 Element: Immobilization/mortality Test Levels: Test concentrations included control, 78, 130, 220, 360, 600and 1000 mg/L. Fifteen daplmia were used per test group (5 per replicate).
Results	48-h EC_{50} = 340 mg/L. 48-h EC_0 = 78 mg/.
Remarks	Measured concentration: n/a Unit: mg/L

	Other: • Effect concentrations based on nominal loading rates. • Control response was satisfactory (100% survival and no sublethal effects).
Conclusions	48-h EC_{50} = 340 mg/L. 95% Confidence limits = (280 – 430) mg/L. 48-h EC_0 = 78 mg/L
Data Quality	*Reliable with restrictions- Klimisch Code 2*
References	*Acute toxicity of OS # 26935F to Daphnia magna. Toxicity test report submitted to Lubrizol Corporation. Bionomics Study # 043-0983-H76-1 IO; Bionomics Report # B W-83- 11 - 1489. EG&G Bionomics, November, 1883.*
Other:	

ENVIRONMENTAL FATE – BIODEGRADATION	
Test Substance	
Chemical Name	2-Acrylamido-2-methylpropanesulfonic acid
CAS #	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS@ category
Method	
Method/Guideline followed	The Evaluation of the biodegradation of test materials using the semi-continuous activated sludge method. (40 CFR 795.3340)
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1986
Contact time (units)	44 days
Inoculum	Secondary activated sludge from domestic wastewater treatment plant.
Remarks for test conditions	Inoculum: Four liters of secondary activated sludge were collected from a domestic waste water treatment plant. Raw sewage collected from the primary settling tank was used as the influent during acclimation and test periods. Concentration of test chemical: From day 1 to 8, 20-mg carbon/L influent was introduced into the test unit. This was reduced to 10-mg carbon/L from day 8. No organic solvents were used to facilitate the dispersion of the test material. The test substance was weighed onto a teflon coupon and introduced into the medium Test Setup: Aeration units consisted of glass vessels with cone shaped lower ends. The units contain 150 ml of mixed liquor when filled. A drain hole is located at the 50 ml level to facilitate sampling and removal of effluent. The acclimation process included aeration for 23 hours, settling of sludge, removing 100 ml of supernatant and resuming aeration to 500 mg/minute. The units were fed raw sewage daily till a clear supematant liquor was obtained. At the end of the acclimation period, sludge from all units was pooled and mixed. Adequate amount was added to each unit to achieve a suspended solids concentration of 1500 mg/L for each unit. One-mg carbon/l00 ml influent was added to the appropriate test units. Blank units did not receive any test material. The study was extended to 44 days to determine if acclimation and biodegradation will increase in this time frame. Sampling frequency: Samples were collected for DOC analysis on all workdays. Controls: Yes; blank unit was included.

	Analytical method: DOC analysis was conducted after sparging the samples with nitrogen prior to analysis to remove soluble carbon dioxide. Method of calculating measured concentrations: N/A Other:
Results	
Degradation % after time	<10% after 44 days.
Kinetic (for sample, positive and negative controls)	n/a
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
Conclusions	The test substance showed a low biodegradation rate (<10%) in *28* days.
Data Quality	*Reliable with restrictions- Klimisch Code 2*
References	*Evaluation of the biodegradation of OS 61349E using the modified SCAS method for Lubrizol Corporation. Ref: 86-0844-I I; Hill Top Research Inc., Nov 1 I, 1986.*
Other:	

ACUTE TOXICITY – ORAL	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-s
Purity:	*99.85%*
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	OECD 401
Type:	Acute oral toxicity study in rats
GLP:	Yes
Year:	1 9 8 1
Species:	Rat'
Strain:	Sherman-Wistar
Route of administration:	Oral
Dose levels:	500, 1000, 2000, 4000 and 8000 mg/kg
Sex and number/group:	5 male rats per treatment group
Frequency of treatment:	Single oral gavage
Duration of test:	14 day observation post-treatment
Control group:	No negative control group
Result	LD50 = 1830 mg/kg (Confidence Limits = 990-3390 mg/kg)
Remark:	Five groups of five male rats of the Sherman-Wistar strain weighing between 200-300 grams were employed in this study. The rats were deprived of food but not water overnight prior to dosing. Each animal was weighed and given a single dose by direct administration of the experimental material in to the stomach by means of a syringe and dosing needle. The sample was dosed as a 50% w/v suspension in water. The following dosages were administered: 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 4000 mg/kg and 8000 mg/kg. Following administration the animals were allowed food and water *ad libitum* for the 14-day observation period during which time the animals were observed for signs of toxicity and mortality. At the lowest dose tested, the animals were lethargic and ruffled after 2 hours. Their condition appeared essentially unchanged after 24 hours. The rats appeared normal within 48 hours. No unscheduled deaths occurred in this group. Animals receiving 1000 mg/kg were lethargic, ruffled and drooling after 1 hour. They were severely depressed after 3 hours. Deaths occurred in 2 animals after 4 hours. The surviving animals were semi-comatose after 24 hours. These animals remained moribund for the next 2-3 days during which time an additional death occurred. The two remaining animals in this group recovered after day 7-8. Animals receiving 2000 mg/kg were lethargic, ruffled and drooling after 30 minutes.

	They were severely depressed after 2 hours. Three animals died 3 hours after dosing. The surviving animals remained severely depressed or semi-comatose for several days before recovery at 7-9 days. Animals receiving 4000 mg/kg were severely depressed, ruffled and drooling 30 minutes after administration of the test article. Two animals died after 3 hours. The remaining animals were semi-comatose for the next 72 hours, with additional two animals expiring on days 3 and 4. The sole surviving animal recovered by day 9. All animals receiving 8000 mg/kg died within 15 minutes of test material administration. No remarkable gross pathological findings were observed. The LD50 was calculated employing the Thompson moving average method as modified by Weil.
Reference	Summary of results of acute toxicology studies: OS61349, Biosearch Inc., 4/28/81.
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

ACUTE TOXICITY – ORAL	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid, sodium salt
CASRN:	5 165-97-g
Purity:	50% aqueous solution
Remarks	This substance is referred to as sodium AMPS@ in the test plan for the AMPS@ category
Method	OECD 401
Type:	Acute oral toxicity study in rats
GLP:	Yes
Year:	1 9 8 1
Species:	Rat
Strain:	Sprague-Dawley albino
Route of administration:	Oral
Dose levels:	1000, 2000, 4000, 8000 and 16000 mg/kg
Sex and number/group:	5 male rats per treatment group
Frequency of treatment:	Single oral gavage
Duration of test:	14 day observation period post-treatment
Control group:	No negative control group
Result	LD50 > 16000 mg/kg
Remark:	Five groups of five male rats of the outbred Sprague-Dawley strain weighing between 200-300 grams were employed in this study. The rats were deprived of food but not water overnight prior to dosing. Each animal was weighed and given a single dose by direct administration of the experimental material in to the stomach by means of a syringe and dosing needle. The following dosages were administered: 1000 mg/kg, 2000 mg/kg, 4000 mg/kg, 8000 mg/kg and 16000 mg/kg. Following administration the animals were allowed food and water ad libitum for the 14-day observation period during which time the animals were observed for signs of toxicity and mortality. No unscheduled deaths were recorded and no unusual clinical or behavioral signs were observed in animals receiving dosages ranging from 1000-8000 mg/kg. Animals receiving 16000 mg/kg appeared ruffled and lethargic within 3-4 hours of test material administration, All animals appeared normal by day 5. No unscheduled deaths were recorded and gross examination revealed no pathological findings.
Reference	Summary of results of acute toxicology studies: OS48933E, Biosearch Inc., 12/17/81.
Data Quality	Valid without restriction (Klimisch Code 1)
Other	

ACUTE TOXICITY – ORAL	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS@ category
Method	OECD 401
Type:	Acute oral toxicity study in rats
GLP:	Yes
Year:	1993
Species:	Rat
Strain:	Sprague-Dawley albino
Route of administration:	Oral
Dose levels:	Limit study at 5000 mg/kg
Sex and number/group:	5 male and 5 female rats per treatment group
Frequency of treatment:	Single oral gavage
Duration of test:	14 day observation period post-treatment
Control group:	No negative control group
Result	LD50 > 5000 mg/kg
Rem ark	One group consisting of five male and five female rats of the Sprague-Dawley strain weighing between 200-300 grams were employed in this study. The rats were deprived of food but not water overnight prior to dosing. Each animal was weighed and given a single dose of 5000 mg/kg by direct administration of the experimental material in to the stomach by means of a syringe and dosing needle. Following test material administration the animals were allowed food and water *ad Zibitum* for the 14-day observation period during which time the animals were observed for signs of toxicity and mortality. There were no unscheduled deaths during the study. Clinical findings were limited to abnormal defecation (soft stool, diarrhea) for three rats and a single occurrence of wet yellow urogenital staining on the day of dosing. One male had dried red material around the nose on day 2. There were no other clinical findings. There were no remarkable changes or differences in body weight during the study. One male had mottled lungs at the terminal necropsy. There were no test material-related gross necropsy findings for any examined tissues at terminal necropsy.

Reference	Acute oral toxicity study of OS# 87613M in albino rats. WIL Research Laboratories, 12/15/93.
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

ACUTE TOXICITY – DERMAL	
Test substance:	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS® in the test plan for the AMPS@ category
Method:	OECD 402
Type:	Acute dermal toxicity in rabbits
GLP:	Yes
Year:	1996
Species:	Rabbit
Strain:	New Zealand albino
Route of administration:	Single dose dermal application
Dose levels:	2000 mg/kg limit test
Sex and number/group:	5 male and 5 female rabbits per treatment group
Frequency of treatment:	Single 24 hour dermal application
Duration of test:	14 day observation period post-treatment
Control group:	No negative control group
Result	LD50 > 2000 mg/kg
Remarks:	One group consisting of five male and five female albino rabbits of the New Zealand strain weighing between 2000-2500 grams were employed in this study. Each animal was weighed and given a single dermal application (24-hour, semi-occluded exposure) of test article at a dose level of 2000 mg/kg. Following administration the animals were observed for 14-days for signs of toxicity and mortality. There were no unscheduled deaths during the study. Two rabbits had instances of soft stool on days 1 and 6, but this finding was determined not to be related to the test material. There were no other clinical findings. The test material induced very slight-to-slight erythema on all animals. Three rabbits had very slight edema. Desquamation was also observed on three rabbits. There were no other dermal findings. All edema subsided by day 2. All dermal irritation completely subsided by day 11 or earlier. There were no remarkable changes or differences in body weights. Upon terminal necropsy, two rabbits displayed accessory splenic tissue, a common congenital abnormality in New Zealand white rabbits. Dark red lungs were noted in two animals. There were no other remarkable findings.
Reference	Acute dermal toxicity study of OS# 114454 in albino rabbits. WIL Research Laboratories, 4/15/96.
Data Oualitv	Valid without restriction – (Klimisch Code 1)

Other	

:N VITRO GENETIC TOXICITY	1
Test substance	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS@ category
Method	OECD 471
Type:	Bacterial reverse mutation assay
Year:	1984
GLP:	Yes
System of testing:	S. typhimurium strains: TA98, TA100, TA1535, TA1537, TA1538
Concentration:	0, 30, 100, 300, 1000 and 3000 microgram/plate
Metabolic activation:	With and without
Results	Non-mutagenic
Remarks:	Concentrations for the mutagenicity assay were chosen on the basis of results from a range finding assay that showed the beginning of toxicity to be approximately 2000-6000 micrograms/plate. Thus, five concentrations of test material were prepared for use as described above. Each concentration was tested in duplicate in the referenced Salmonella strains, both in the presence and absence of a S9 rat liver metabolic activation system. Bacterial strains were checked the day of each assay to ensure that all five strains were sensitive to crystal violet (rfa mutation) and the TA98 and TAlOO contained the R-factor (resistance to ampicillin). The maximum amount of test material used (3000 microgram/plate) was toxic to all strains in the assay. No mutagenic response was seen for stains TA98, TA100, TA1535 or TA1537 in either the activated or non-activated assays. Non-activated test material did produce a small increase in the number of revertants per plate, however, this increase was deemed to be the result of an unusually low number of revertant colonies on the corresponding negative control plates. The mutagenicity test was repeated to confirm the previous observations. The known mutagens used in the positive control experiments (2-anthramine, sodium azide, 2-nitrofluorene or 9-aminoacridine depending of the bacterial strain) produced significant increases in the frequency of revertants (approximately 40-fold over the DMSO-containing vehicle control). In conclusion, the test material did not induced significant increases in mutations in strains of S. typhimurium, either in the presence or absence of metabolic activation, up to toxicity limits. Thus, within the limitations of this assay, the test material was considered to be non-mutagenic.

Reference	Salmonella/microsome mutagenesis assay on OS# 61349B. Bioassay Systems Corp., 4/10/84.
Data Quality	Valid without restriction -- (Klimisch Code 1)
Other	

IN VITRO GENETIC TOXICITY	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	OECD 47 1/472
Type:	Bacterial reverse mutation assay
Year:	1991
GLP:	Y
Type:	Bacterial reverse mutation assay
System of testing:	S. typhimurium strains: TA98, TA100, TA1535, TA1537, TA1538, E. coli WP2
Concentration:	0, 15, 50, 150, 500, 1500 and 5000 micrograms/plate
Metabolic activation:	With and without
Results	Non-mutagenic
Remarks:	Four strains of S. typhimurium and 1 strain of E. coli were used in the assays. The bacterial cultures were tested for genetic characterization (rfa mutation or ampicillin resistance) on the day of the assays. The test material was applied in an aqueous vehicle to bacteria at concentrations ranging from 15-5000 microgram/plate. Negative controls (sterile water or DMSO) or positive controls (2-aminoanthracene, 9-aminoacridine, sodium azide, N-ethyl-N'-nitro-N-nitrosoguanidine, 2-nitrofluorene depending on the bacterial strain and activation parameters) were used to verify the reliability of the assay. The metabolic activation system consisted of an S9 microsomal fraction obtained from the liver harvested from rats 5 days following a single intraperitoneal injection of Aroclor 1254. The mean number of revertants per plate and the standard deviation was calculated for each concentration and strain. A test result was considered positive if for any strain, a significant increase over the negative control in the number of revertants per plate was observed as concentration-dependent; or after a two-fold increase when the background was 50 revertants per plate or greater; or after a three-fold increase when the background was between 10 and 49 revertants per plate; or after a four-fold increase when the background was less than 10 revertants per plate. Each individual assay was performed in triplicate. The test substance was slightly toxic to all Salmonella strains at the highest dose used (5000 microgram/plate) both with and without metabolic activation. For all bacterial strains tested there was no significant increase in the number of revertants at any dose of test material when compared to the

	corresponding negative solvent control. The entire assay was repeated and the results seen in the first experiment were confirmed. The positive and control solvent control for all experiments were within established historical ranges for each bacterial strain utilized. In conclusion, the test substance did not produce a significant increase in the number of revertants, with and without metabolic activation. Thus, at the concentrations tested and under the conditions of the assay, the test material was considered to be non-mutagenic.
Reference	Evaluation of OS61 349H in a bacterial mutagenesis assay. A.D. Little Inc., 7/23/91.
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

IN VITRO GENETIC TOXICITY	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS@ category
Method	OECD 476
Type:	Mammalian Cell Gene Mutation Test
Year:	1991
GLP:	Y
System of testing:	Chinese hamster ovary cells
Concentrations:	Geometric increments from 130 – 4000 microgram/ml
Exposure period:	16 hours
Metabolic activation:	Without
Control and treatment:	The negative control was water vehicle and the positive control was ethylmethanesulfonate
Results	Non-mutagenic
Remarks:	The objective of the study was to assess the ability of the test material to induce mutations in CHO at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus. CHO cells obtained from the Oak Ridge National Laboratory were maintained in Ham's nutrient F 12 medium supplemented with 10% fetal bovine serum, 50 U/ml penicillin, 50 microgram/ml streptomycin and 2 mM glutamine. These mycoplasma-free cells were grown at 37°C in 5% CO2 and 95% relative humidity. For testing, cells were seeded at a density of 5 x 10^5 cells/l00 mm tissue culture plate, and 24 hours later were treated in triplicate with either test material (400 mg/ml aqueous stock solution) or quality control preparations. The negative control was water vehicle and the positive control was ethylmethanesulfonate (235 microgram/ml). Experimental plates were cultured in the presence of non-activated test material ranging from 130-4000 microgram/ml for 16 hours. After that time period, medium containing the test material was removed, the cells detached under trypsin, washed and sub-cultured for 9-12 days to allow phenotypic expression. At the end of the phenotypic expression period, the cells were again sub-cultured in hypoxanthine-free Ham's F12 medium containing 10% fetal bovine serum and 10 uM thioguanine (five plates per test material concentration). These plates were incubated for 7-9 days, at which time the cells were fixed in methanol, stained in 5% Giemsa, dried and the mutant colonies counted. The HGPRT mutation frequency was calculated by dividing the total number of mutant colonies by the total number of cells seeded per

	culture (corrected for cloning efficiency). Preliminary range finding experiments revealed the test material to be toxic to CHO cells at concentrations of 2000 microgram/ml (50% cell loss) and 4000 microgram/ml (100% cell loss). The entire assay was repeated twice. At the concentrations tested, the assay material did not increase the frequency of mutant cells when compared to the negative vehicle control. In the first assay the mutation frequencies per 10^6 cells exposed to test material ranged from 13.2 to 78.3 and in the second assay the frequencies resulting from test material application ranged from 17.5 to 47.0 per 10^6 cells exposed. The mean mutation frequency for a comparable number of exposed cells exposed to water vehicle was 35.6 and 42.4, respectively. There was no statistically significant difference between the mutation frequencies at any dose of test material and the values obtained from the vehicle control (i.e., water). The positive control ethylmethanesulfonate produced mean mutation frequencies of 1308 and 241 per10^6 cells exposed. The responses obtained from the negative and positive controls demonstrated that the system was capable of detecting mutagenic chemicals. Therefore, the experimental results indicate that the non-activated test material was non-mutagenic in CHO cells under the conditions of the assay.
Reference	CHO/HGRPT in vitro mammalian cell mutation assay without metabolic activation with OS 6 1349H. A.D. Little, Inc. 12/10/91.
Data Quality	Valid with restriction – (Klimisch Code 2) – The mutagenesis experiments performed with a metabolic activation system did not yield reliable results because the CHO cells did not grow. The assays were repeated, and the results were submitted in a separate report (see Mammalian Cell Gene Mutation Test below).
Other	

IN VITRO GENETIC TOXICITY	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance **is referred to** as AMPS® acid in the **test** plan for the AMPS@ category
Method	OECD
Type:	Mammalian Cell Gene Mutation Test
Year:	1992
GLP:	Y
System of testing:	Chinese hamster ovary cells
Concentrations:	Geometric increments from 1 O-5000 microgram/ml
Exposure period:	19 hours
Metabolic activation:	With and without
Control and treatment:	The negative control was water vehicle and the positive controls were ethylmethanesulfonate (235 microgram/ml) in the non-activated experiments and dimethylnitrosamine (100 microgram/ml) in the activated experiments
Results	Non-mutagenic
Remarks:	The objective of the study was to assess the ability of the test material to induce mutations in CHO at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus. CHO cells obtained from the Oak Ridge National Laboratory were maintained in Ham's nutrient F12 medium supplemented with 5% fetal bovine serum, 50 U/ml penicillin, 50 microgram/ml streptomycin and 2 mM glutamine. These Mycoplasma-free cells were grown at 37°C in 5% CO2 and 90% relative humidity. For testing, cells were seeded at a density of 1 x 10^6 cells/100 mm tissue culture plate, and 24 hours later were treated in duplicate with either test material (400 mg/ml aqueous stock solution) or quality control preparations. CHO cells were assayed in the absence and in the presence of Aroclor 1254-induced male Sprague-Dawley rat liver homogenate supplemented with cofactors (S9). The negative control was water vehicle and the positive controls were ethylmethanesulfonate (235 microgram/ml) in the non-activated experiments and dimethylnitrosamine (100 microgram/ml) in the activated experiments. Assay plates were cultured in the presence of activated and non-activated test material (500 mg/ml aqueous stock solution) ranging from 1 O-5000 microgram/ml for 19 hours. After that time period, medium containing the test material was removed, the cells detached under trypsin, washed and sub-cultured for 7 days to allow phenotypic expression. At the end of the phenotypic expression period, the cells were again sub-cultured in

hypoxanthine-free Ham's F 12 medium containing 5% fetal bovine serum and 10 microM thioguanine (five plates per test material concentration). These plates were incubated for 8 days, at which time the cells were fixed in ethyl alcohol, stained in a dilute solution of crystal violet, dried and the mutant colonies counted. The HGPRT mutation frequency was calculated by dividing the total number of mutant colonies by the total number of cells seeded per culture (corrected for cloning efficiency). A test article was considered to be a mutagen if it; a) exhibited a dose-dependent increase in the average mutant frequencies (least-squares method of linear regression analysis; $p < 0.05$), with at least one concentration producing a statistically significant increase in average mutant frequency that was greater than or equal to three times the average mutant frequency of the pooled concurrent negative controls, or b) represented a net increase of 20 mutants per 10^6 clonable cells. Preliminary range finding experiments revealed the test material to be toxic to CHO cells at concentrations > 3000 microgram/ml with and without metabolic activation. At the concentrations tested, the assay material did not increase the frequency of mutant cells when compared to the negative vehicle control. In the first experiment, the mutant frequencies of the negative control cultures ranged from 10.7 to 17.3 (mean 14.2, s.d. 6.9) survivors per 10^6 clonable cells. The cultures treated the test material (with and without metabolic activation) ranged from 4.6 to 33.0 mutants per 10^6 clonable cells. There were no statistically significant or dose-dependent increases in the average mutant frequencies of the cultures treated with either activated or non-activated test material. The test material was re-evaluated in a confirmatory assay under identical conditions. In these experiments, the average mutant frequencies of the negative cultures ranged from 4.5 to 33.9 (mean 19.2, s.d. 15.8) mutants per 10^6 clonable cells while those of the cells treated with activated or non-activated test material ranged from <2.5 to 29.1 mutant per 10^6 clonable cells. Again, there were no statistically significant or dose-dependent increases in the average mutant frequencies of the cultures treated with either activated or non-activated test material. The reliability of the assay for detecting mutagens was demonstrated by the observation that average mutant frequencies for the activated and non-activated positive controls were 334.5 and 275.0 survivors per 10^6 clonable cells. These results indicate that the test material, whether activated or non-activated, was non-mutagenic in CHO cells under the conditions of the assay.

Reference	CHO/HPRT Mammalian Cell Forward Mutation Assay on OS 6 13495. Pharmakon Research International, Inc., 2/7/92
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

IN VITRO GENETIC TOXICITY	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS® acid in the test plan for the AMPS' category
Method	OECD 473
Type:	Mammalian cytogenetic test
Year:	1991
GLP:	Y
System of testing:	Chin&e hamster ovary cells
Concentrations:	6 geometric increments ranging from 0 to approximately 6000 microgram/ml (dependent on exposure period and activation treatments)
Exposure period:	10 and 20 hours
Metabolic activation:	With and without
Controls and treatment:	Water vehicle as a negative control, and mitomycin C or cyclophosphamide as a positive controls
Results	Clastogenic after metabolic activation

Non-Activated				Activated			
Time (hrs)	Treatment	Dose (ug/ml)	% cells with aberrations	Time (hrs)	Treatment	Dose (ug/ml)	% cells with aberrations
10	Water		4.5	10	Water		2.7
	OS61349H	510	5.5		OS61349H	177	5.6
		1530	3.0			539	7.7*
		5100	7.0			1790	5.5
	Mitomycin C	0.3	36.0			3530	10.5*
20	Water		2			5890	NR
	OS61349H	344	2		Cyclophosphamide	50	46.2
		1150	1.5	10 (repeat)	Water		4.0
		3440	2		OS61349H	151	3.0
		5730	NR			505	6.3
	Mitomycin C	0.3	76			1510	6.0
						3010	10.3*
						5010	4.7
					Cyclophosphamide	50	40.0
				20	Water		4.5
						189	5.5
* = p < 0.05 compared to negative control						630	1.5
NR = Aberrations not scored due to cytotoxicity						1890	6.5
CT = evidence of cytotoxicity						6300	51.0* CT
					Cyclophosphamide	50	96
				20 (repeat)	Water		1.5
						1010	4.5
						2010	3.5
						3010	3.5
						4010	1.0
						5010	6.0*
					Cyclophosphamide	50	68

Table 1.

Remarks:	Chinese hamster ovary (CHO) cells (Merck Institute for Therapeutic Research) were maintained in McCoy's 5A nedium containing 10% fetal bovine serum, 50 units/ml penicillin, 50 microgram/ml streptomycin, 2mM glutamine, buffered with NaHC03, and incubated under 95% relative humidity at 37°C and 5% C02. The cells were determined to be free from mycoplasma contamination. Non-activated assay: CHO cells were seeded at a density of 5 x 10^5 in 25 cm^2 culture flasks. The following day, the cells were exposed to media containing the test material (50% aqueous solution) at approximately 153,306, 510, 1020, 1530, 3060 or 5 100 microgram/ml for a duration of either 10 or 20 hours. Separate flasks were prepared containing either water vehicle as a negative control, or 0.3 microgram/ml mitomycin C as a positive control. All test sample concentrations and controls were tested in duplicate flasks. Two hours prior to harvest, vinblastine was added to 0.26 microgram/ml to arrest the cells in metaphase. At the end of this period, metaphase cells were collected with trypsin and concentrated by centrifugation. The cells were then washed in PBS, lysed in hypotonic KCL and sodium citrate and fixed in methanol:acetic acid (3:1). Drops of each lysate were dried on glass slides and stained with 5% Giemsa. Activated assay: CHO cells prepared similarly were exposure to test or control material for two hours in the presence of S9 microsomal fractions were prepared from the liver harvested from rats treated 5 days earlier with a single injection of Aroclor 1254 (500 mg/kg). In the activated experiments, cyclophosphamide at 50 microgram/ml was used as a positive control. Following this exposure, the cells were washed, re-incubated to complete the 10 or 20 hour time period and treated as described above. Mitotic index was determined on a minimum of 500 metaphase cells exposed to test material. At least 100 metaphase cells from a minimum of three selected test sample concentrations were analyzed for chromosomal aberrations. The data was analyzed using the statistical methods described by Margolin et al., Environ. Mutagen 8: 183,204, 1986. Non-activated results (Table 1): The test material was toxic to CHO cells at 5730 microgram/ml in the 20-hour non-activated assay, whereas in the lo-hour assay, no toxicity was observed. In the 20-hour non-activated study, the mitotic index (%) averaged 18.4 and 15.2 at 344 and 1150 micrograms/ml, respectively. At 3440 micrograms/ml the mitotic index fell to 9.5%. The mitotic index for pure water solvent averaged 15.9%. In the 1 O-hour non-activated study, the mitotic index (%) averaged 4.5 and 5.6 at 510 and 1530

	micrograms/ml, respectively. At 5 100 micrograms/ml the mitotic index fell to 3.4%. The mitotic index for pure water solvent averaged 4.5%. None of the test material concentrations evaluated (344-2440 microgram/ml) caused a statistically significant increase in the number of chromosomal aberrations compared to vehicle controls after either exposure period. Activated results (Table 1): The test material was also assayed 10 and 20 hours after microsomal activation. Toxicity was observed at the highest concentrations tested (approximately 6000 microgram/ml) after 10 and 20 hours. The mitotic indices (%) for the 10-hour activated assay were as follows: Study 1 – Pure water solvent (1 1), 177 microgram/ml (8.9), 589 microgram/ml (9.5), 1790 microgram/ml (14.5), 3530 microgram/ml (7.9) and 5890 microgram/ml (0.2). Study 2 – Pure water solvent (8.1), 151 microgram/ml (5.0), 505 microgram/ml (4.9), 1510 microgram/ml (3.6), 3010 microgram/ml (5.6) and 5010 microgram/ml (6.4). The mitotic indices (%) for the 20-hour activated assay were as follows: Study 1 Pure water solvent (10.6), 189 microgram/ml (10.2), 630 microgram/ml (19.5), 1890 microgram/ml (7.5), and 6300 microgram/ml (2.3). Study 2 – Pure water solvent (5.3), 1010 microgram/ml (6.2), 2010 microgram/ml (5.3), 3010 microgram/ml (6.1) 4010 microgram/ml (4.2) and 5010 microgram/ml (6.0). The activated test material produced statistically significant chromosomal damage in CHO cells after 10 hours at approximately 3 000 micrograms/ml and after 20 hours at concentrations of 5010 and 6300 microgram/ml. Based upon these results, the Study Director concluded that the activated test material was clastogenic in Chinese hamster ovary cell culture. However, this finding is confounded by the absence of a dose-response effect, lack of a time-response effect, lack of reproducibility between repeat experiments and the presence of extensive cytotoxic damage concurrent with observed chromosomal damage for one scored time point.
Reference	The Evaluation of OS 61349H in the In Vitro Chromosomal Aberration Assay. A.D. Little, Inc. 1 1/4/91.
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

IN VIVO GENETIC TOXICITY	
Test substanc:	2-Acrylamido-2-methylpropanesulfonic acid
CASRN:	15214-89-8
Purity:	99.85%
Remarks	This substance is referred to as AMPS@ acid in the test plan for the AMPS@ category
Method	OECD 475
Type:	Bone marrow cytogenetics test
Year:	1992
GLP:	Yes
Species:	Rat
Strain:	Sprague-Dawley
Sex:	5 male and 5 female rats per treatment group
Route:	Oral gavage
Exposure period:	6, 18 and 24 hours
Doses:	150,500 and 1500 mg/kg
Controls and treatment:	Concurrent vehicle negative control (water), positive control animals treated with cyclophosphamide
Results:	Non-clastogenic

Male				Female			
Time (hrs)	Treatment	Dose (mg/kg)	% cells with aberrations	Time (hrs)	Treatment	Dose (mg/kg)	% cells with aberrations
6	Vehicle		3.2	6	Vehicle		2.0
	OS61349J	150	0.8		OS61349J	150	2.0
		500	1.6			500	0.8
		1500	1.2			1500	1.2
18	Vehicle		0.4	18	Vehicle		0.4
	OS61349J	150	0.4		OS61349J	150	1.2
		500	0.4			500	0.8
		1500	0.8			1500	1.2
24	Cyclophosphamide	30	18.8	24	Cyclophosphamide	30	19.2
	Vehicle		0.8		Vehicle		1.6
	OS61349J	150	0.0		OS61349J	150	1.2
		500	0.8			500	2.4
		1500	0.8			1500	0.8

Table 2.

Remarks	The test material solution was administered to 8-9 week old test animals by oral gavage at a single dose of 150, 500 or 1500 mg/kg using plastic syringes and stainless steel intubation needles. The animals were not fasted prior to dosing. Six males and six females were dosed per group to ensure that acceptable metaphase cells could be obtained from 5 animals per group. Fifty metaphase cells from each of 5 animals per test condition were analyzed when possible. The total number of cells analyzed per dose at each exposure period equaled 250. Exposure time included 6, 18 and 24 hours. Clinical signs observed in the animals

Remarks	The test material solution was administered to 8-9 week old test animals by oral gavage at a single dose of 150,500 or 1500 mg/kg using plastic syringes and stainless steel intubation needles. The animals were not fasted prior to dosing. Six males and six females were dosed per group to ensure that acceptable metaphase cells could be obtained from 5 animals per group. Fifty metaphase cells from each of 5 animals per test condition were analyzed when possible. The total number of cells analyzed per dose at each exposure period equaled 250. Exposure time included 6, 18 and 24 hours. Clinical signs observed in the animals dosed at 1500 mg/kg included increased respiratory rate (50%), watery feces (28%), soft feces (17%), and wheezing (1%). Mean body weights of the dose groups were not significantly different from the vehicle control group at any time point. As is illustrated in Table 1 above, the test article did not produce statistically significant increases ($p<0.05$) in the percentage of cells with aberrations at any dose, time period or for either sex, compared to control values. Positive control animals treated with cyclophosphamide (30 mg/kg) demonstrated an increase in the frequency of damaged cells which was statistically significant at the study threshold of $p < 0.05$. Thus, the positive and negative controls demonstrated the reliability of the assay to detect chromosomal aberrations. The percentage of cells with chromosomal aberration were analyzed by the statistical methods described by Margolin et al., Environmental Mutagenesis, Volume 8, 1986. These results indicate that the test material was non-clastogenic in rat bone marrow cells under the conditions of the assay.
Reference	Evaluation of OS6 1348J in the in vivo chromosomal aberration assay. A.D. Little Inc., 1/7/92
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

IN VIVO GENETIC TOXICITY	
Test substance	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-g'
'Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS@ in the test plan for the AMPS@ category
Method	OECD 474
Type:	Micronucleus assay
Year:	1996
GLP:	Y
Species:	Mouse
Strain:	Crl:CD-1 (ICR)BR
Sex:	Five male and five female mice/dose/time period
Route:	Intraperitoneal
Exposure period:	24, 48 and 72 hours
Doses:	175,875 and 1750 mg/kg
Controls and treatment:	Concurrent vehicle negative control (water), positive control animals treated with cyclophosphamide
Results	The test material was considered to be non-clastogenic in the mouse micronucleus test, under the conditions and according to the criteria of the test protocol.

Male				Female			
Time (hrs)	Treatment	Dose (mg/kg)	% MPCE Frequencies	Time (hrs)	Treatment	Dose (mg/kg)	% MPCE Frequencies
24	Vehicle		0.03	24	Vehicle		0.03
	OS114454	175	0.07		OS114454	175	0.08
		875	0.03			875	0.04
		1750	0.02			1750	0.05
	Cyclophosphamide	60	2.24		Cyclophosphamide	60	1.39
48	Vehicle		0.04	48	Vehicle		0.05
	OS114454	175	0.01		OS114454	175	0.08
		875	0.04			875	0.03
		1750	0.03			1750	0.07
72	Vehicle		0.03	72	Vehicle		0.05
	OS114454	175	0.04		OS114454	175	0.05
		875	0.07			875	0.02
		1750	0.07			1750	0.03

Table 3.

Remarks	The test material solution was administered to ~ 7 week old test animals by intraperitoneal injection at a single dose of 175, 875 or 1750 mg/kg at a constant volume of 10 ml/kg. Nine groups of mice were used to evaluate the induction of micronuclei (five male and five female mice/dose/time period). Negative control (deionized water vehicle) and positive control (cyclophosphamide; 60 mg/kg) were administered concurrently for sacrifice at all three time

PCE/NCE Ratios		
Dose (mg/kg)	Time (hours)	Ratio Mean (sd)
H2O	24	1.217 (0.3)
175	24	1.265 (0.12)
875	24	1.294 (0.17)
1750	24	1.256 (0.19)
H2O	48	1.374 (0.22)
175	48	1.369 (0.31)
875	48	1.317 (0.23)
1750	48	1.422 (0.24)
H2O	72	1.281 (0.11)
175	72	1.266 (0.20)
875	72	1.364 (0.27)
1750	72	1.133 (0.25)

Table 4.

Remarks	The test material solution was administered to ~ 7 week old test animals by intraperitoneal injection at a single dose of 175, 875 or 1750 mg/kg at a constant volume of 10 ml/kg. Nine groups of mice were used to evaluate the induction of micronuclei (five male and five female mice/dose/time period). Negative control (deionized water vehicle) and positive control (cyclophosphamide; 60 mg/kg) were administered concurrently for sacrifice at all three time points. Groups were sacrificed after 24, 48 and 72 hours, with the exception of the control groups, which were sacrificed at 24 hours. Bone marrow slides were prepared, stained and scored for the number of micronucleated polychromatic erythrocytes (MPCE) in a total of 2000 polychromatic erythrocytes (PCE) per mouse. The ratio of polychromatic to normochromatic erythrocytes (PCE/NCE ratio) was also determined for each mouse as an index of toxicity. The frequency of MPCE in each test group was compared to its respective negative control group using a one-tailed Student's t-test with a post hoc Cochran-Armitage test for possible dosing trends. Analysis of the by-sex data indicated that the test material did not induce any statistically significant or dose-dependent increases in MPCE frequencies at any harvest time evaluated, as compared to concurrent negative controls (Table 3). However, a single isolated increase in the frequency of MPCEs (combined-sex) was observed in mice treated with the test material at a dose of 175 mg/kg and harvested at 24 hours ($p < 0.05$). Since this increase was within the historical negative control range as well as the acceptable negative control values for this assay, this isolated increase in MPCE frequency was considered a statistical aberration due to a random fluctuation of the spontaneous MPCE frequency. In addition, the test material did not induce any statistically significant depressions in PCE/NCE ratios compared to concurrent negative controls (Table 4). MPCE frequencies for all negative control groups were within acceptable negative ranges, and the cyclophosphamide positive control groups produced statistically significant increases in MPCE frequencies (combined- and by-sex; $p < 0.01$). Cyclophosphamide-treated animals experienced significant depressions in PCE/NCE ratios ($p < 0.05$). In conclusion, the test material was considered to be non-clastogenic in the mouse micronucleus test, under the conditions and according to the criteria of the test protocol.

Reference	In vivo micronucleus test with OS# 114454 in mouse bone marrow erythropoietic cells. Pharmakon USA, 7/3/96.
Data Quality	Valid without restriction -- (Klimisch Code 1)
Other	

REPEAT DOSE TOXICITY	1
Test substance	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	58.4% aqueous solution
Remarks	This substance is referred to as ammonium **AMPS@** in the test plan for the AMPS® category
Method	OECD 407
Type:	Repeated dose oral toxicity in rats
Year:	1995
GLP:	Y
Species:	Rat
Strain:	Sprague-Dawley [Crl:CD (SD)BR] 44 days old'at initiation of dosing
Sex:.	10 male and 10 female rats per group
Route of Administration:	Oral gavage
Exposure Period:	28 days
Frequency of Treatment:	7 days/week
Post-exposure observation period:	14 days
Dose:	0, 50, 150,400 or 1000 mg/kg
Control group:	Concurrent negative control group treated with water
NOEL:	1000 mg/kg/day
Results	Analysis of the test material confirmed the homogeneity and stability of the sample preparations. All animals survived throughout the study. With the exception of a single high dose male, there were no remarkable clinical observations resulting from oral administration of the test material. One male receiving 1000 mg/kg/day exhibited a number of adverse signs (lethargy, emaciation, watery stool, yellow ano-genital staining, and decreased fecal volume) during the first week of the study but recovered by the end of week 2. No unusual postmortem findings were seen in this particular animal at study termination. Mean body weights and body weight gains for the 1000 gm/kg/day male group were slightly lower than control values at week 1 (not statistically significant). Values for the control and treated groups of males were comparable from weeks 2 through 4. Body weight values for control and treated females were comparable throughout the study. Food consumption in the male 1000 mg/kg/day group was transiently lower during week 1. Values in subsequent weeks were similar to, or higher than, control values. No effect on food consumption was seen in with any other dose either in the male or female groups. No effects of test material administration on

	hematology values were evident after four weeks of treatment or after the 2-week treatment-free recovery period (including but not limited to hemoglobin concentration, hematocrit, RBC count, platelet count, MCV, MCH, MCHC, white cell differential, PTT, aPTT, RBC morphology). No effects of test material administration on clinical chemistry indices were evident during the treatment or recovery phases of the study (including but not limited to electrolytes, liver enzymes, plasma proteins, serum lipids, glucose, creatinine and BUN). Urinalyses were unremarkable (i.e., protein, glucose, ketone, occults blood, pH, bilirubin, urobilinogen, appearance, specific gravity). No effects of test material administration were observed on organ weights (i.e., adrenal glands, brain, kidneys, liver, ovaries, testes) for any dose, sex or phase of the study. None of the macroscopic findings in rats sacrificed at the end of the treatment and post-treatment recovery periods were considered to be related to treatment with the test material. No test material-related changes in microscopic findings on 29 various organs or tissues were observed for any group. Mean values of all dose groups were compared to the mean value for the control group at each time interval. Statistical evaluation was made by the appropriate one way analysis of variance technique followed by a post-hoc comparison procedure (e.g., Dunnett's test or Kruskal-Wallis test). Oral gavage administration of the test material to rats at doses of 50, 150,400 and 1000 mg/kg/day produced no significant toxicity at any dose level. Therefore, under the conditions of this study, the No Observed Effect Level (NOEL) was determined to be 1000 mg/kg/day.
Reference	A 4-week Toxicity Study of OS 14454 in the Rat via Oral Gavage Administration Followed by a 2-week Recovery Period. Huntingdon Life Sciences, 7/9/96.
Data Quality	Valid without restriction – (Klimisch Code 1)
Other	

REPRODUCTION TOXICITY	
rest substance:	2-Acrylamido-2-methylpropanesulfonic acid, ammonium salt
CASRN:	58374-69-9
Purity:	50% aqueous solution
Remarks	This substance is referred to as ammonium AMPS@ in the test plan for the AMPS@ category
Method	OECD 421
Type:	Repeated dose oral reproductive/developmental toxicity screen in rats
Year:	2000
GLP:	Yes
Species:	Rat
Strain:	Sprague-Dawley [Crl:CD (SD)BR] - Approximately nine weeks of age at initiation of the study
Sex:	12 male and 12 female rats per group
Route of Administration:	Oral gavage
Frequency of Treatment:	7 days/week
Dose:	0, 100, 500, or 1000 mg/kg
Control group:	Concurrent with water vehicle
Pre-mating exposure period for males:	14 days
Pre-mating exposure period for females:	14 days
Reproductive and Developmental NOEL:	F0 'g&&&&ion NOEL = 1000 mg/kg/day F 1 generation NOEL = 1000 mg/kg/day
Remarks	Chemical analyses demonstrated that the test article was homogenous and stable at the concentrations used in the study. Dosing preparations were administered by oral gavage, as a single daily dose to FO males and females beginning two weeks prior to mating. The FO males were dosed for approximately seven weeks, including the two weeks prior to mating, during mating and through post-mating. The FO females were dosed throughout the study, including the two weeks prior to mating, during mating, during gestation, and following parturition. Following 14 days of treatment with test material or vehicle control, each FO female was cohabitated with a single FO male randomly selected from the same treatment group. The day of confirmed copulation was designated as day 0 of gestation and the female was returned to its cage. If no evidence of copulation was observed after the 14 day mating period, the female was separated from the male and the mating phase was concluded. At the termination of the study periods, all surviving FO males and females were euthanized in a

humane fashion and subjected to gross necropsy examination. The FO males were euthanized after approximately seven weeks of treatment. FO females that umderwent parturition were euthanized on lactation day 4. F0 females that failed to deliver were euthanized 25 days after evidence of mating. FO females with no evidence of mating were euthanized 25 days after completion of the mating period. The necropsy examination on FO males and females included evaluation of the external surfaces of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. Uterine contents were examined and the number of implantation sites and number of corpora lutea (per ovary) were recorded. Uteri with no macroscopic evidence of implantation were opened and placed in 10% aqueous ammonium sulfide solution for detection of early embryo lethality. Testes of all FO males were preserved in Bouin's fixative. Gross lesions, ovaries, prostate, epididymides, seminal vesicles uterus and vagina from all FO animals were preserved in formalin for future histopathological examination. Statistical analysis was performed using ANOVA with Dunnett's post-hoc test, Chi-square test, and Mann-Whitney U test where appropriate for each specific endpoint. Analytical chemistry results indicated that the test material was homogenous and stable at the concentrations used in the study. **Subchronic effects:** Oral administration of the test material at doses of 100,500 and 1000 mg/kg/day had no effect on FO survival, and there were no remarkable clinical observations noted during the study. **Reproductive Indices, Precoital Intervals and Gestation Lengths:** There were no statistically significant or toxicologically meaningful differences in copulation or fertility indices among the groups. The copulation index was 100% in the control and the 1000 mg/kg/day groups, and 91.7% in the 100 and 500 mg/kg/day groups. The fertility index was 100% in the control, 100 and 1000 mg/kg/day groups, and 8 1.8% in the 500 mg/kg/day. No statistically significant differences were observed in group mean precoital intervals or gestation lengths. Mean precoital intervals in the control, 100, 500 and 1000 mg/kg/day groups were 1.5, 2.8, 3.5 and 2.6 days, respectively. Mean gestation lengths in the control, 100, 500 and 1000 mg/kg/day groups were 22.1, 22.0, 21.8 and 22.1 days, respectively. **FO Gross Necropsy Observations:** Gross necropsy finding were generally unremarkable. When anomalies were observed, they were of low incidence, randomly distributed among the groups, and were not considered to be treatment related. **FO Organ Weights:**

	There were no statistically significant or toxicologically neaningful differences in absolute or relative testes and epididymides weights between the control and test material-reated groups. **FO Histopathology:** Histopathological examination of the testes, ovaries and epididymides from control and high-dose rats did not reveal any test material--elated microscopic changes. **FO Implantation and k-e/Post-Implantation Loss:** There were no statistically significant or toxicologically meaningful differences between control and test material-treated groups with respect to corpora lutea counts, implantation scar counts, mean number of live pups, or pre- or post-implantation loss. **F1 Pup Viability:** There were no toxicologically meaningful differences with respect to F 1 pup viability, number of litters in each group with live offspring, mean live litter size or pup sex ratios. The incidence of dead pups on lactation day 0 was slightly higher (statistically significant) in the 500 mg/kg/day group. This difference was not considered toxicologically significant since a dose-response pattern was not demonstrated (i.e., the incidence of dead pups was comparable between the vehicle control and the 1000 mg/kg/day groups). **F1 Pup Observations during Lactation:** F1 pup observations during lactation were generally unremarkable with any individual findings randomly distributed among the groups and without dose-responsive pattern. **F1 Pup Body Weights during Lactation:** There were no statistically significant or toxicologically meaningful differences in F1 pup body weights during lactation. **F1 Pup Gross Necropsy Observations:** Among the F1 pups found dead or euthanized as scheduled on lactation day 4, gross necropsy did not reveal any findings which would indicate a relationship to treatment with the test material. The most notable gross observation in pups found dead on lactation day 0 consisted of atelectasis and absence of milk in the stomach, indicating that these pups were likely stillborn. Other necropsy findings tended to be of low incidence and randomly distributed among the groups. There were no indications of treatment-related developmental effects.
Conclusion	The Study Director assigned a dosage level of 1000 mg/kg/day as the no observed effect level (NOEL) for this reproduction and developmental screening study in rats.
Reference	An oral (gavage) reproduction/developmental toxicity screening study in Sprague-Dawley rats with OS#132086. Springbom Laboratories Inc., 3/15/00.
Data Quality:	Valid without restriction – (Klimisch Code 1)

Other	